Effects of carbon substrate and irrigation on carbon dioxide emissions and denitrification for three grassland soils

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Yuan Li

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Conversion of non irrigated grassland to high−intensity farm systems with irrigation is a major land−use change to enable a higher yield year−round in New Zealand. Research is needed to better understand the links between soil carbon (C), water and nitrogen (N) and their dynamics in order to minimise the impacts of farm management practices on losses of soil C and N. The aim of the research was to investigate carbon dioxide (CO_2) and nitrous oxide (N_2O) emissions in relation to physical factors, and/or C and N substrate dynamics under irrigated grassland. Ultimately, the research will contribute to developing optimal irrigation management practices that promote the retention of soil organic matter (SOM) while minimising C and N losses. The key hypothesis of this study is that irrigation induced changes to soil water content will promote plant growth and thus root exudation, while also affecting relative gas diffusivity, CO_2 emissions and N_2O formation mechanisms. The research was based on experimental laboratory work oriented towards understanding soil C−N interactions in order to mitigate CO_2 and N_2O emissions in grazed grasslands.

The aim of the first experiment was to determine the impacts of C substrates on soil CO_2 and N_2O emissions, under varying soil types and soil water contents. Three repacked Pallic grassland soils containing NO_3^--^{15}N were held at three levels of matric potential (ψ, −3, −5 and −7 kPa), while receiving daily substrate additions (acetate, glucose, water control) for 14 days. The daily CO_2 and N_2O emissions were monitored. Additionally, the N_2O:(N_2+N_2O) ratios were determined using ^{15}N methods on days 3 and 14. Results showed that across all soils, N_2O peak emissions were higher for soils treated with glucose, with a range (± SD) of 0.1 ± 0.0 to 42.7 ± 2.1 mg N m^{-2} h^{-1}. The highest cumulative N_2O emission (2.5 ± 0.2 g N m^{-2}) was measured in glucose-treated soil at a ψ of −3 kPa. In comparison with added glucose, acetate resulted in 2-fold higher N_2 emissions in soils at low diffusivities. The N_2O:(N_2O+N_2) emissions ratios varied with soil type (0.91-0.80) on day 3. Cumulative CO_2 emissions increased with increasing soil diffusivity and soils amended with glucose had higher cumulative CO_2-C emissions, ranging from 22.5 ± 1.3 to 36.6 ± 1.8, g C m^{-2}. Collectively, I demonstrated that the increase of N_2O, N_2 and CO_2 emissions in response to acetate or glucose

addition depended on both soil type and soil matric potential. The findings indicate that non-fermentable substrates will enhance denitrification from soil.

Using a similar setup and treatments, the aim of the second experiment was to determine the relationships between the priming effect and N_2O emissions from the soil, in relation to N and C supply. I applied ^{13}C–labelled substrates (acetate, butyrate, glucose; 80 µg C g^{-1}, 6 atom% excess ^{13}C), with water as a control, and ^{15}N–labelled N as KNO_3 (300 µg N g^{-1} soil, 40 atom% excess ^{15}N) to three different soils and, after 3 days, measured the effects on the priming of SOM and sources of N_2O emissions. I demonstrated that C substrate addition increased both CO_2 and SOM derived N_2O emissions in the presence of exogenous N. Emissions of CO_2 and N_2O from soils with added glucose (0.73 ± 0.13 µmol m^{-2} s^{-1} and 21.4 ± 12.1 mg N m^{-2} h^{-1}) were higher than those from soils treated with acetate (0.64 ± 0.11 µmol m^{-2} s^{-1} and 10.9 ± 6.5 mg N m^{-2} h^{-1}) or butyrate (0.61 ± 0.11 µmol m^{-2} s^{-1} and 11.0 ± 6.6 mg N m^{-2} h^{-1}), respectively. Acetate addition induced a stronger priming effect (0.07 ± 0.09 µmol m^{-2} s^{-1}) than that for glucose (0.02 ± 0.10 µmol m^{-2} s^{-1}), while butyrate addition resulted in negative priming (-0.09 ± 0.05 µmol m^{-2} s^{-1}). SOM derived N_2O emissions were relatively low from soils with butyrate addition (1.4 ± 1.5 mg N m^{-2} h^{-1}) compared with acetate (2.9 ± 2.3 mg N m^{-2} h^{-1}) or glucose (9.2 ± 4.5 mg N m^{-2} h^{-1}). However, I did not detect a clear relationship between priming effect and SOM derived N_2O emissions. The findings highlight the need to consider the nature of the C substrate when interpreting processes regulating SOM decomposition and N_2O emission source.

In the third experiment, the components of net ecosystem C balance (F_N) were partitioned for a C_4 plant Bermuda grass (*Cynodon dactylon L.*), growing in mesocosms and irrigated with the same total quantity of water (15 mm day^{-1}) applied at intervals of 1, 2, 3 days for 12 days (treatments I_1, I_2, and I_3, respectively), whereafter treatment I_2 was changed to watering every 6 days (treatment I_6) and treatments I_2 and I_3 were continued for a further 18 days. Daily measurements of evaporation were made by weighing the mesocosms and chambers were used to measure rates of CO_2 exchange to estimate F_N, ecosystem respiration and respiration from leaves and soil plus roots (R_S), and gross C uptake by the plants. Further, use of the C_4 plant enabled partitioning of R_S into the autotrophic and heterotrophic components of belowground respiration using a ^{13}C natural abundance isotopic technique, requiring destructive sampling at the end of the experiment when differences in cumulative soil water deficit between the treatments were greatest. The findings showed that, over short periods with well–drained soil, irrigation frequency could be managed to manipulate soil water deficits to reduce net belowground respiratory C losses, particularly those from the microbial decomposition of SOM, with no significant effects on biomass production and N_2O emissions. Thus, changes to the scheduling of irrigation could reduce CO_2 emissions and SOM decomposition but not N_2O emissions in conditions of moderate to high water deficits.

This study showed that CO_2 and N_2O emissions are dependent on C availability and diffusivity that accounts for differences in both soil water content and soils, but there is no clear relationship between priming of SOM and N_2O emissions. Changes to the scheduling of irrigation could be used to minimise soil C losses but that this is unlikely to affect N_2O emissions with low N inputs on well-drained soils.

Keywords: carbon dioxide emissions, nitrous oxide emissions, C_4 grass, natural abundance ^{13}C, net ecosystem carbon exchange, relative soil gas diffusivity, stable isotopes (^{13}C and ^{15}N)

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Part of this thesis has been submitted for publication. The article in the scientific journal based on the results presented in this thesis is listed below:

1. Chapter 5 has been submitted to Pedosphere (manuscript ID pedos201909626). Effects of irrigation frequency on the components of ecosystem carbon balance and nitrous oxide emissions for a C_4 grassland growing in mesocosms. Yuan Li, Gabriel Y. K. Moinet, Timothy J. Clough, John E. Hunt, David Whitehead

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List of key symbols and abbreviations

C	Carbon
C_A	Carbon input through photosynthesis
C_I	Carbon input to the ecosystem
C_O	Carbon output from the ecosystem
C_M	Gain of the ecosystem C through management practices
C_E	Gain t of the ecosystem C through ruminant livestock excreta
C_B	Loss of the ecosystem C through harvested and grazing biomass
C_L	Loss of the ecosystem C through leaching
C:N	Ratio of C to nitrogen
CO_2	Carbon dioxide
C_{soil}	CO_2 emissions from soils treated with C substrates
DOC	Dissolved organic C
D_P/D_O	Soil relative gas diffusivity
F_N	Net ecosystem CO_2 exchange
F_G	Gross primary production
N	Nitrogen
N_2	Dinitrogen gas
N_I	Nitrogen input to the ecosystem
N_O	Nitrogen output from the ecosystem
N_F	Gain to ecosystem N through fertiliser or effluent
N_A	Gain to ecosystem N through atmospheric deposition or biological N fixation
N_D	Gain to ecosystem N through ruminant livestock excreta
N_G	Loss from ecosystem N through gaseous losses
N_B	Loss from ecosystem N through harvested and grazing biomass
N_L	Loss from ecosystem N through soil runoff, erosion and leaching
N_2O	Nitrous oxide
NH_3	Ammonia
NH_4^+	Ammonium
NO_2^-	Nitrite
NO_3^-	Nitrate
O_2	Oxygen
P_C	Carbon priming effect
$P_{C,r}$	P_C expressed as a percentage of CO_2 emissions from the control treatments
N_r	Reactive N

N_{total}	Total N_2O emissions
N_{som}	N_2O emissions derived from SOM
N_F	N_2O emissions derived from added N fertiliser
R_A	Autotrophic respiration rate
R_E	Ecosystem respiration rate
R_H	Heterotrophic respiration rate
R_L	Leaf respiration rate
R_s	Soil respiration rate
SOC	Soil organic C
SOM	Soil organic matter
TN	Total N
W	Water deficit
WFPS	Water−filled pore space
WHC	Water holding capacity
θ_g	Gravimetric water content
θ_v	Volumetric water content
ρ_b	Bulk density
ψ	Matric potential

Chapter 1 Introduction

1.1 Background

In New Zealand, one of the major land−use changes in recent decades has been the dramatic increase in the number of intensive dairy farms (MacLeod and Moller, 2006). Over the last decades, there has also been an increase in the intensity of these dairy farming systems (MacLeod and Moller, 2006). Furthermore, the intensification of farming has occurred through land use change. Sheep farm conversions to dairy farms have occurred, typically in the South Island. To maintain the required grassland productivity, high intensity farm management practices have been introduced that include irrigation and enhanced use of nitrogen (N) fertiliser, often as urea (Monaghan et al., 2007). Irrigation increases productivity per unit of land per year, by maintaining an optimum soil water content during seasonally dry periods (Siebert et al., 2015). Three surveys of water use in 1999, 2006 and 2010 showed that the total volume of water allocated nationally had increased by a third over an 11 year period, mainly due to an increase in the area of irrigated land, especially in Canterbury and Otago (Feltham, 2011). However, there remain large uncertainties with respect to the environmental sustainability of such intensification (Schipper et al., 2017; Whitehead et al., 2018). Concerns regarding intensification include the potential for losses of soil carbon (C) and N from the soil profile after dryland soils are converted to irrigated dairy farming, with associated higher N inputs (Schipper et al., 2007; Mudge et al., 2017; Whitehead et al., 2018).

In New Zealand, grassland ecosystems occupy 40% of the total land surface (MacLeod and Moller, 2006), and represent 50% of the national C stock (Tate et al., 2005). Evidence from the limited long−term data that are available suggests that soil C stocks decrease or remain unchanged, decades after conversion to dairy farming (Mudge et al., 2017). Other studies have shown net C uptake on intensively managed dairy farms that, if sustained over time, could result in increasing soil C stocks (Mudge et al., 2011; Hunt et al., 2016). A better understanding of the mechanisms leading to changes in irrigated grassland C and N dynamics is required to determine whether intensive dairy systems are sustainable in terms of C and N stocks.

Irrigation can alter soil C and N cycles, leading to an imbalance between photosynthetic inputs, and rates of C and N mineralisation and, in some cases, favoring the release of the greenhouse gases carbon dioxide (CO_2) and nitrous oxide (N_2O) (Schipper et al., 2007; Trost et al., 2013). Irrigation and addition of fertiliser not only change soil C and N cycling dynamics but they also influence soil water

content and subsequently soil oxygen (O_2) concentration, which may also affect CO_2 and N_2O emissions (Linn and Doran, 1984; Owens et al., 2017). Globally, N_2O currently comprises ~6% of the greenhouse effect, with N_2O having a global warming potential ~300 times stronger than that for CO_2 over a 100 year time frame (Ciais et al., 2013). The rising concentration of N_2O in the atmosphere is predominantly a consequence of anthropogenically induced agricultural emissions (Ciais et al., 2013), hence the need to mitigate the emissions.

Collectively, further research is required to understand the drivers and linkages between soil C, N dynamics and water to determine the impacts of intensifying farm systems on losses of soil C and N.

1.2 Thesis objectives

The overall objective of this research was to investigate the mechanisms and regulators of soil CO_2 and N_2O (N_2) emissions in irrigated grasslands. The three objectives were

Objective 1. To determine the effects of adding daily inputs of C, either glucose or acetate, on N_2O, N_2, and CO_2 production in repacked cores of three different soils held at varying soil matric potentials (Chapter 3).

Objective 2. To determine the effects of different sources of C substrate (i.e. glucose, organic acids) in combination with N, as nitrate, on the direction and magnitude of SOM priming and the partitioning of N_2O emission sources (Chapter 4).

Objective 3. To determine the effects of irrigation frequency, in particular, the water deficit, on the relationship between net ecosystem CO_2 exchange rate, gross C uptake, and ecosystem respiration from plants and soil (Chapter 5).

1.3 Thesis structure

This thesis is divided into 6 chapters (Fig. 1.1). After an introduction in Chapter 1 that provides an overview of the thesis topic, the relevant literature is reviewed in Chapter 2. Then the following chapters, 3, 4 and 5, present and discuss the experiments undertaken. Finally, chapter 6 summarises the overall findings and recommends future research directions. Chapters 3, 4, and 5 are presented as manuscripts. Specifically:

Chapter 1 provides a high−level introduction to the research objectives, and associated hypotheses.

Chapter 2 presents a literature review that concentrates on outlining the factors affecting soil C and N losses as CO_2 and N_2O emissions within irrigated grassland. This includes a summary of

pathways and factors affecting CO_2 and N_2O emissions in grassland soils, a review of previous studies associated with the effects of C substrates addition and irrigation on soil CO_2 and N_2O emissions from grassland soils, and identified research gaps.

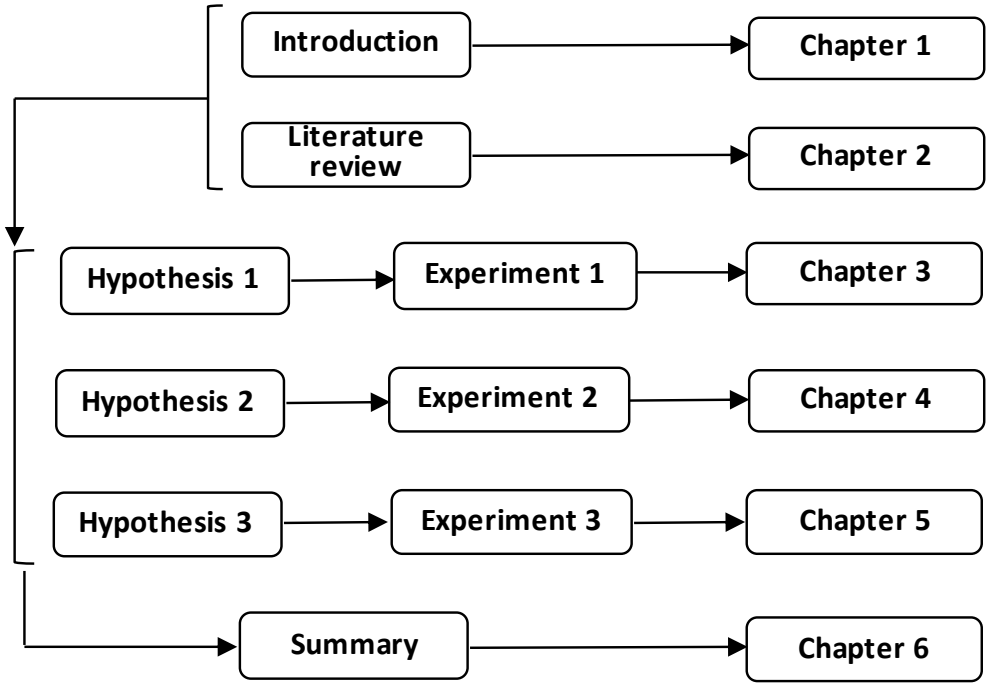

Fig. 1.1 Thesis outline showing links between chapters.

Chapter 3 'Emissions of nitrous oxide, dinitrogen and carbon dioxide from three soils amended with carbon substrates under varying soil matric potential' reports on CO_2 and N_2O emission dynamics from three soils held at varying moisture contents (−3 to −7 kPa) while receiving varying C substrates.

Chapter 4 'Nitrous oxide emissions from denitrification depend on the energy available from soil organic matter decomposition and added carbon substrates' leads on from chapter 3 and reports on the effect of C substrate type, in the presence of nitrate, on soil CO_2 and N_2O emissions, and the direction and magnitude of SOM priming and the partitioning of N_2O emission sources. The experimental chapter analysed emissions of $^{13}CO_2$ and $^{15}N_2O$ (and N_2) following the addition of isotopically labelled substrates in order to quantify C priming and origin of the N_2O emissions that were observed in Experiment 1.

Chapter 5 'Effects of irrigation frequency on the components of ecosystem carbon balance and nitrous oxide emissions for a C_4 grassland growing in mesocosms' reports on the effects of varying soil water deficit (irrigation frequency) on the relationship between net ecosystem CO_2 exchange rate, gross C uptake and ecosystem respiration. A natural ^{13}C abundance

technique was used to partition C sources of soil respiration, C_3 soil or C_4 plants, in order to evaluate the effects of induced soil water deficits.

Chapter 6 presents a synthesis of the findings from the experimental chapters and draws overall conclusions with suggestions made for further research.

Chapter 2 Literature review

2.1 Introduction

Due to the increase in atmospheric CO_2, since the early 19th Century, it has become increasingly urgent to understand the global C cycle and particularly the potential role of various C sinks. Soil is the largest terrestrial reservoir of C, with global estimates ranging from 1,115 to 2,200 Pg of C (Batjes and Bridges, 1992), and soils store more than twice the amount of C found in the atmosphere (Batjes, 2014). Increasing the quantity of C stored in agricultural soils has the potential to offset emissions of greenhouse gases to the atmosphere. Conversely, even fractionally small soil C losses would further add to the atmospheric CO_2 loading (Smith, 2008). Grassland ecosystems are a regulator of global C and N budgets because grassland soils act as a reservoir for both organic C and N (McSherry and Ritchie, 2013). Grasslands cover the equivalent of 70% of the world's agricultural surface area (Conant et al., 2011) but soil C losses have occurred with increasing adoption of intensive agricultural practices (Lal, 2004). There is the potential to store a substantial fraction of atmospheric CO_2 as stable C in grassland soils (Reid et al., 2004) due to their high land area. However, despite considerable research over recent decades, much uncertainty still exists regarding the effects of intensification on grassland soil C.

Nitrous oxide is a major greenhouse gas and has a global warming potential of ~300 times greater than that for CO_2 over a 100 year time period (Ciais et al., 2013), and is also involved in the depletion of stratospheric ozone (Ravishankara et al., 2009). Agricultural soils are the dominant anthropogenic N_2O source and contribute 60% of the total anthropogenic N_2O emissions (Ciais et al., 2013). Managed grasslands are known to be significant contributors to the global N_2O budget and N_2O emissions from grazed grasslands are estimated to be approximately 28% of global anthropogenic N_2O emissions (Rafique et al., 2011). Atmospheric N_2O concentrations are expected to double by 2050 (Davidson et al., 2013) so there is an urgent need to identify mitigation options.

This literature review summarises the effects of grassland management on soil C and N stocks with a specific focus on soil C and N losses, as CO_2 and N_2O, when grasslands are irrigated and supplied with N fertiliser.

2.2 The importance of soil carbon in grasslands

Grasslands comprise almost 40.5% (52.5 million km^2) of the global land surface (Conant et al., 2011), and consequently play a significant role in the global C cycle. Grasslands generally have a high inherent SOM concentration that supplies plant nutrients, increases soil aggregation, limits soil erosion, and also increases cation exchange and water holding capacity (Miller and Donahue, 1990). Thus, maintenance of SOM is a key factor in the sustainability of grassland ecosystems.

Grassland contains 12% of Earth's SOM, and the average SOM stored in temperate grasslands is 331 Mg ha^{-1} (Schlesinger, 1977). The upper 1 m of grassland soils contain 303 Pg C, and this is about 20% of the world's total soil C stock (Stockmann et al., 2013). In temperate zones, such as New Zealand, the mean turnover rate of soil organic C (SOC) is 61 years (Raich and Schlesinger, 1992). Grassland SOM turnover can be strongly influenced by management (Conant et al., 2017; Whitehead et al., 2018). A variety of management techniques have been developed to increase forage production for livestock, which have the potential to alter SOC concentration and turnover rate. A review of data from grasslands, assembled from hundreds of studies to document soil C responses to changes in management, confirm that practices such as improved grazing, fertiliser addition, irrigation, and conversion from cultivation, tend to increase soil C stocks, at rates ranging from 1 to > 10 t C ha^{-1} yr^{-1} (Conant et al., 2017).

Further, enhancing soil C concentration is critical for improving soil quality in terms of soil structure and hydraulic properties (Six et al., 2000; Lal, 2004), that in turn enhance agricultural primary production (Lal, 2004). Therefore, more international projects have been emerging, for instance, the '4 per 1000 Initiative: Soils for food security and Climate' (Rumpel et al., 2018), which sets the goal to increase global soil C stocks by 4‰ per year for all land uses.

2.2.1 Carbon balance for irrigated grazed grasslands

Net SOC stocks (C_N) in grassland depend on the relative balance between C inputs and outputs (Equation 2.1), C_I and C_O, respectively (Post et al., 1990; Mudge et al., 2017; Whitehead et al., 2018). Carbon inputs (Equation 2.1) occur via photosynthesis (C_A), the application of effluents, feed import, or other organic materials (C_F), and ruminant livestock excreta (C_D). While outputs (Equation 2.1) include harvested biomass and products transferred off−site (C_E), respiration losses as CO_2 (C_R), losses of C as methane, and losses via leaching as dissolved organic C (C_L). Figure 2.1 represents the relationship between the components of the grazed grassland C balance, where

$$C_N = C_I - C_O = (C_A + C_F + C_D) - (C_E + C_R + C_L). \quad (2.1)$$

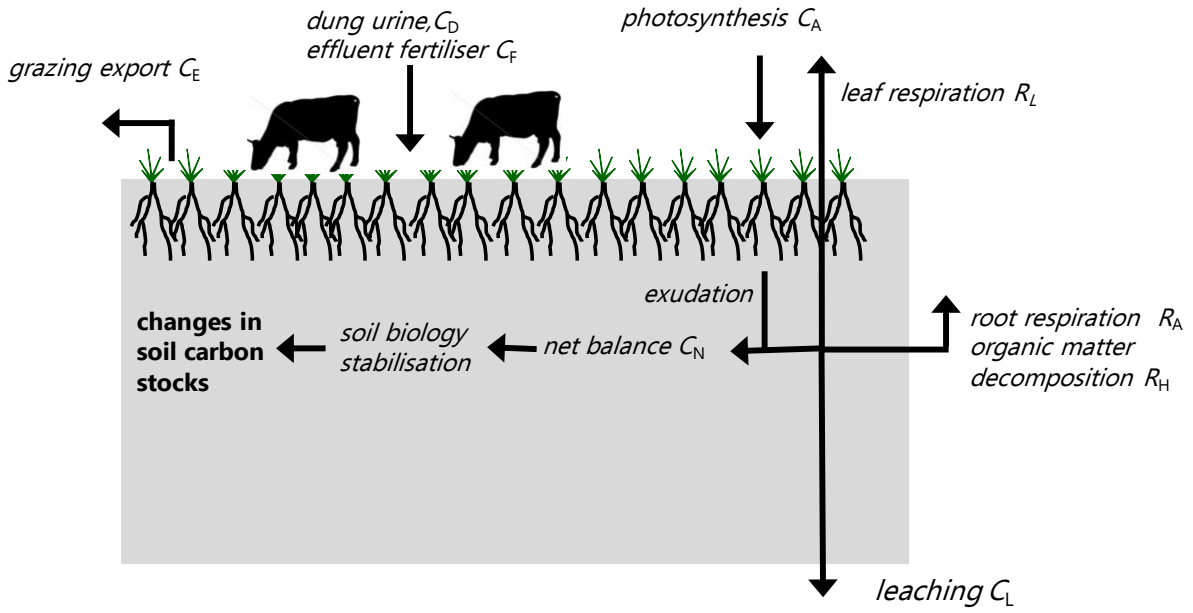

Fig. 2.1 Processes regulating changes in soil carbon stocks in grassland (Whitehead et al., 2018).

Carbon cycling in grasslands begins with photosynthesis that results in the production of organic compounds. Conversely, respiration returns C to the atmosphere (Fig. 2.1) (Post et al., 1990; Whitehead et al., 2018). In grassland, the largest C input is photosynthesis and is termed gross primary production (F_G). As much as 20% of the C fixed by photosynthesis is released into the soil through root exudates (Davidson and Trumbore, 1995). Pausch and Kuzyakov (2018) reported that the total C allocated belowground depends on the plant species. For example, approximately 50% of the total C assimilated by perennial ryegrass (*Lolium perenne* L.) was allocated to the belowground pool 12 hours after the labelling (Domanski et al., 2001). While only 12% of the total assimilated C in annual ryegrass (*Lolium multiflorum* Lam.) was translocated into the soil within 24 hours (Butler et al., 2004).

Ecosystem respiration represents a significant proportion of photosynthetically fixed C and it comprises pathways where C is returned to the atmosphere as CO_2. It has long been known that aboveground processes strongly regulate belowground C inputs and soil respiration (Raich and Nadelhoffer, 1989). Thus, a better understanding of the magnitude of the C inputs and losses in grassland systems could facilitate the development of farm systems which minimise C losses or enhance C uptake (Mudge et al., 2011).

Recently, irrigation has been used increasingly in New Zealand (Feltham, 2011) to increase grassland production during periods of the year where soil water deficits result from evaporation exceeding precipitation, especially during summer periods. For example, in comparison with dryland, irrigation treatments of 260 mm yr^{-1} and 770 mm yr^{-1} increased dry matter production in a temperate grazed grassland by 44 and 74%, respectively (Condron et al., 2014). Reported effects of irrigation on grassland soil C concentration are limited and inconclusive. Irrigation increased soil C stocks by 5.4% when an initially low fertility Conargo sandy loam soil in New South Wales, Australia was irrigated over 5 years (Rixon, 1966). In a deep sandy grassland soil under continuous dairy cattle grazing in the Canterbury region of New Zealand, Kelliher et al. (2015) assessed the 0−0.3 m soil depth and found that the soil C concentration was higher for the irrigated site (100 ± 3 tonne C ha^{-1}, mean ± standard error) than the value for the non−irrigated site (78 ± 6 tonne C ha^{-1}). Conversely, after analysing 34 paired (irrigated and non-irrigated) grassland sites across New Zealand, Mudge et al. (2017) observed that soil C stocks were lower at irrigated sites compared to those at adjacent non irrigated sites. For example, at a temperate grazed grassland field experiment maintained under different irrigation treatments for 62 years, Condron et al. (2014) found that the SOC concentration in the upper 1 m (93 t ha^{-1}), with irrigation of 770 mm yr^{-1}, was significantly lower than that for a paired dryland site (126 t ha^{-1}). But reasons why this occurred could only be speculated upon: it was suggested that SOC was lower under the irrigation site relative to the non-irrigation site due to increased removal of biomass (Condron et al., 2014; Vogeler et al., 2019), and decomposition of SOC by the soil microorganisms (Brown et al., 2009; Moinet et al., 2016a). Thus, studies on the soil C dynamics and subsequently the ecosystem C balance in irrigated grassland ecosystems are required to better understand the processes that regulate C gains or losses under irrigation.

2.2.2 Respiration in grassland ecosystems

A portion of the organic C fixed via photosynthesis is utilised to supply the plants with energy and is termed plant respiration. This includes aboveground autotrophic leaf respiration (R_L) and belowground autotrophic root respiration (R_A; Equation 2.2; Fig, 2.2). In the soil, heterotrophic organisms utilise SOM to respire, termed R_H. The sum of R_H and R_A equals the total soil respiration (R_S). In turn, the sum of R_S and R_L equate to ecosystem respiration (R_E; Equation 2.2; Fig. 2.2). Ecosystem respiration is dominated by R_S, with R_S representing a large component in the terrestrial global C balance (Raich and Schlesinger, 1992).

$$R_E = R_L + R_S = R_L + (R_A + R_H). \quad (2.2)$$

In a study investigating the regulation of R_S in a typical temperate cattle−grazed grassland in New Zealand, Brown et al. (2009) reported that R_E comprised of 84% R_S and 16% R_L, with rates of R_S responding strongly to water addition. Hence, R_S can vary with irrigation. For example, irrigation ranging from 683 to 1484 mm, increased cumulative R_S by 7 to 49%, respectively, specifically between 257 g C m^{-2} yr^{-1} and 500 g C m^{-2} yr^{-1}, in a native perennial grass (*Elymus nutans*) in a temperate grassland of Inner Mongolia, China (Gong et al., 2015). Figure 2.2 summaries the components of respiration that contribute to R_E for grassland soils.

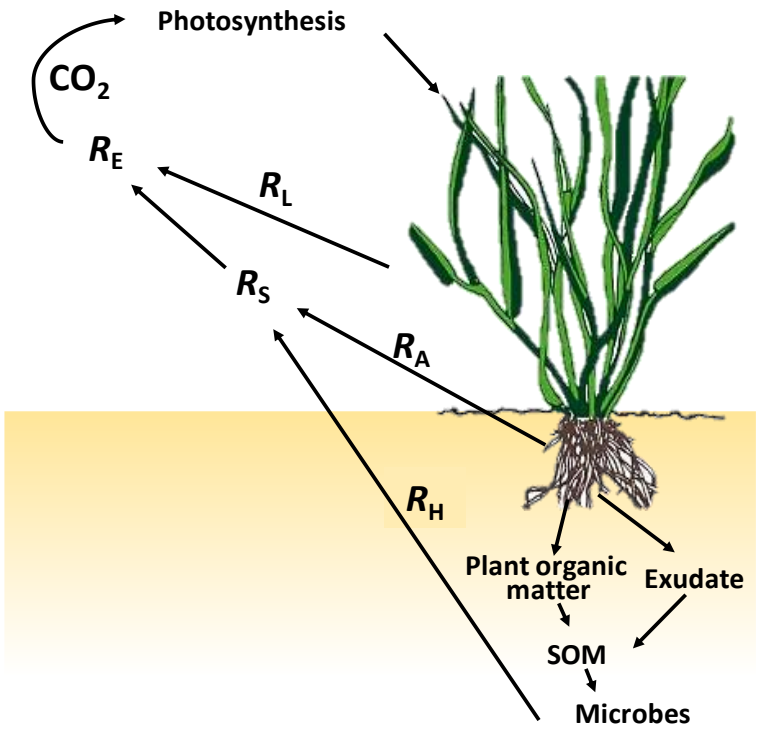

Fig. 2.2 The relationship between the components of ecosystem respiration (R_E) are comprised of the plant autotrophic leaf respiration (R_L) and soil respiration (R_S); in turn, R_S includes heterotrophic microbial respiration (R_H) and plant autotrophic root respiration (R_A).

2.2.2.1 Leaf and root respiration

Root respiration is a major component of R_S, usually accounting for at least 50% (Hanson et al., 2000). Plants allocate between 40−60% of photosynthetically fixed C to roots and associated microorganisms via sloughed−off root cells, tissues, and a variety of exuded organic compounds (Keiluweit et al., 2015). Photosynthetically fixed C has been shown to be rapidly transported from the leaf to the root. In a laboratory experiment using *Lolium perenne* on a loamy Gleyic Cambisol soil, R_A accounted for between 1.5 and 6.5% of the photosynthetically assimilated C over 8 days (Kuzyakov et al., 1999). Using a ^{14}C pulse−labelling technique to determine seasonal changes in

assimilation and partitioning of photoassimilate C in the plant−root−soil components of a temperate grassland, it was found that 1.2−4.0% of the C uptake by leaves was transferred into the soil within 4 hours (Saggar and Hedley, 2001). Total plant respiration, including R_L and R_A, is thus largely dependent on photosynthesis and plant biomass (Lloyd and Farquhar, 2008). A study of 11 temperate mountain grasslands, including meadows, grasslands, and abandoned sites at three geographic locations reported that R_A showed distinct seasonal changes due to changes in root biomass, with fine roots contributing the largest portion of R_A, which was 35−96% of R_A (Bahn et al., 2006). Root production in grassland ecosystems is strongly influenced by the aboveground biomass, while the relationship between leaf and root growth is dynamic due to seasonal effects, plant growth stage, and managerial practices (Kuzyakov et al., 1999; Saggar and Hedley, 2001; Xu et al., 2017). Thus, soil water availability plays a significant role in determining R_A as water influences plant photosynthesis and thus the rate of C allocation (Huxman et al., 2004). For instance, using a natural abundance ^{13}C technique in an undisturbed C_3 plant dominated temperate grassland, Moinet et al. (2016a) found that irrigation strongly increased R_A during spring to summer, with R_A positively correlated with the soil water content.

2.2.2.2 Heterotrophic respiration in grassland soils

Deposition of organic debris from or associated with plant roots during periods of active growth is termed rhizodeposition, and as such the soil adjacent to the root presents a favourable habitat for soil microorganisms, and is termed the rhizosphere (Shamoot et al., 1968). Microbes in the rhizosphere decomposing the recently added C substrates are heterotrophic microbes (Kuzyakov, 2006). In a laboratory experiment using perennial ryegrass with ^{14}C−pulse labelling, Kuzyakov et al. (1999) found that 2.0 to 8.0% of the photosynthetically assimilated C was allocated to the rhizosphere within 8 days.

A large proportion of the SOM is stabilised and protected from physical, chemical, and/or biochemical decomposition (Six et al., 2000), and thus the rate of SOM decomposition is much slower than that of root−derived C. The size of the SOC pool is far larger than the size of the root−derived C in the soil (Gaudinski et al., 2000). In other words, the contribution of SOM decomposition to R_S is relatively low when plants are present. However, the SOC pool is considerably larger than the root−derived C pool and so interest in changes in the rate of SOM decomposition, in the context of agricultural intensification, has increased because of its importance to the global C cycle (Trost et al., 2013; Schipper et al., 2017; Whitehead et al., 2018). Nonetheless, the response of SOM decomposition to irrigation remains unclear. For example, a meta−analysis of data associated with different types of grassland reported that irrigation increased R_H by 28% (Zhou et al., 2016).

While in an undisturbed perennial temperate grassland dominated by perennial ryegrass and white clover (*Trifolium repens* L.), it was found that R_H was insensitive to irrigation and remained constant over a six month period during spring and summer (Moinet et al., 2016a).

Addition of substrates to soil affects the rate of SOM decomposition, either positively or negatively (Fig. 2.3). Priming effects are strong short−term changes in the turnover of SOM caused by comparatively moderate treatments of the soil (Fig. 2.3) (Parnas, 1976; Kuzyakov et al., 2000). In the course of priming effects, a large amount of C, N and other nutrients can be released from or immobilised in the soil over a very short time (Kuzyakov et al., 2000; Cheng et al., 2014). Plant−soil interactions therefore play a central role in terrestrial ecosystem functions, and these interactions often occur in the rhizosphere (Cheng et al., 2014). In general, C compounds released by roots have an important effect on the priming effect (Cheng et al., 2014). For example, using substrates found commonly in root exudates, Keiluweit et al. (2015) demonstrated that oxalic acid promotes C loss by enhancing microbial access to previously mineral-protected C compounds.

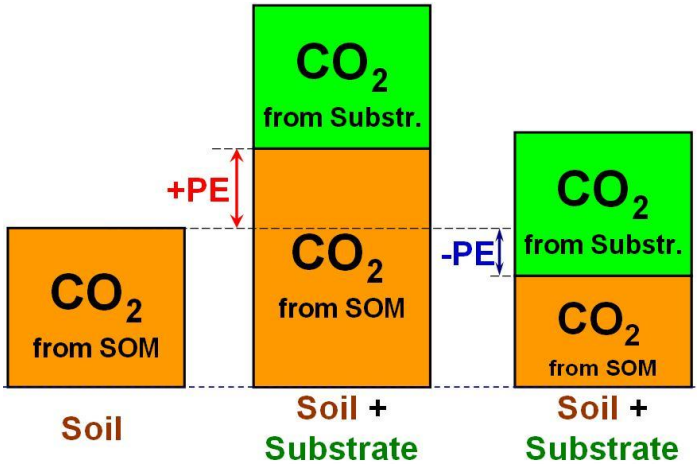

Fig. 2.3 Schematic diagram of the priming effect, strong short−term changes in the turnover of SOM caused by comparatively moderate treatments of the soil, showing the relative decomposition of added substrate and soil organic matter (SOM): increase in SOM decomposition−positive priming effect (middle); decrease in SOM decomposition−negative priming effect (right) (Kuzyakov et al., 2000).

It has been acknowledged that biological factors are more important than the physical factors in terms of their effects on priming (Kuzyakov et al., 2000). This is explained by both the growth stage of plants and the magnitude of photosynthesis affecting the intensity of priming (Kuzyakov and Cheng, 2004; Cheng et al., 2014). For example, the absence of light suppressed the priming effect as

root−derived C decreased due to the absence of photosynthesis in wheat (*Triticum aestivum* L.) (Kuzyakov and Cheng, 2001).

The direction and magnitude of priming have also been shown to depend on the chemical nature of C substrates within soils (Cheng et al., 2014). Organic acids, such as oxalic acid, have been observed to result in stronger positive priming than glucose due to the solubilisation of mineral−protected SOM (Keiluweit et al., 2015). The nature of C substrates affects the availability and accessibility of the substrates for microbes (Bore et al., 2019), and thus they have various effects on the priming effect. For example, despite glucose yielding more energy per unit C for microbes than acetate (Paul et al., 1989; Gunina et al., 2014), glucose has a weaker priming effect due to the lower dissolution of mineral−associated C than that of acetate (Keiluweit et al., 2015; Yuan et al., 2018). In addition, the intensity of priming and thus R_H may also be influenced by soil type as differences in the microbial community composition, or initial C fraction between soils may alter the supply of C (Kuzyakov et al., 2000; Joergensen and Wichern, 2018). For example, when ^{13}C labelled common vetch (*Vicia sativa* L.), pea (*Pisum sativum* L.), or wheat were applied to either a sandy-loam or clay soil, it was found that the priming effect varied with the quality of the crop residue, soil type, and their interaction (Schmatz et al., 2017). Additionally, different soil types have differing native SOM concentrations and native microbial communities (Morley et al., 2014), and soil water content directly affects decomposition processes (Sommers et al., 1981) and gas diffusion (Balaine et al., 2013; Balaine et al., 2016) in the soil. Thus, the effect of additional C substrate on CO_2 emissions is highly dependent on the sources of the C substrate, the soil type, and soil water content. However, there remains a need to determine the priming effects of C substrates under various soil conditions.

2.2.2.3 Respiration and net ecosystem CO_2 exchange

Net ecosystem CO_2 exchange (F_N) is the difference between gross carbon uptake (F_G) and ecosystem respiration from plants and soil (R_E; Equation 2.3) as

$$F_N = F_G - R_E. \quad (2.3)$$

The terminology adopted is for positive values of F_N to indicate net uptake of CO_2 by the ecosystem. On a global scale, annual F_G is about ~120 Pg C. Approximately half of the CO_2 (60 Pg C) taken up during photosynthesis is used in plant fixation and the other half is respired back to the atmosphere by plants (Chapin et al., 2011). Annually, global R_E is estimated to be 117 Pg C (Chapin et al., 2011). To put this into the context of a grazed temperate grassland the study of Rutledge et al. (2017) provides a good example. Following the renewal of an 80-year old perennial ryegrass grassland, mean annual F_G and R_E were 22.4 and 21.2 t C ha^{-1}, respectively, so the mean F_N was 1.2 t C ha^{-1}. In

general, F_G and R_E showed a similar seasonal pattern to grassland production, with rapid reductions in F_G and R_E taking place as the soil dried out from mid-summer. When low soil water availability limited grassland productivity, grassland became a modest source of CO_2 with $R_E > F_G$. The ecosystem responded to rainfall events with large emissions of CO_2 such that daily F_N was −0.1 t C ha^{-1} d^{-1}. While a review on soil C change in New Zealand grazed grasslands by Schipper et al. (2017) reported that increases in gross C inputs in grassland ecosystems did not lead to increases in soil C stocks. The authors recommended that, considering the widespread conversion of dry stock to dairy farming in New Zealand, more research is required to investigate the impacts of irrigation on grassland soil C stocks. While some studies have compared soil C stocks between irrigated and non-irrigated grasslands (Mudge et al., 2011; Condron et al., 2014; Kelliher et al., 2015; Mudge et al., 2017), there has been less emphasis on the effects of managing the frequency and intensity of irrigation.

2.2.3 Measurements to partition the components of soil CO_2 emissions

2.2.3.1 Non−isotopic techniques

There are various methods for partitioning the components of CO_2 emissions from soil, specifically R_A and R_H. Non−isotopic techniques are used commonly by comparing emissions from planted and unplanted soil commonly termed the 'root exclusion technique' (Kuzyakov, 2006). However, a major criticism of this method is that soil temperature and water content usually differ between the treatments (Fisher and Gosz, 1986) and that R_H is different in the presence and absence of roots (Kuzyakov, 2006).

2.2.3.2 Natural ^{13}C abundance stable isotope technique

The natural abundance ^{13}C isotope technique to partition the source of soil CO_2 emissions into R_A and R_H has an advantage over root exclusion methods that it avoids disturbance effects.

The technique is based on the discrimination of the heavier $\delta^{13}C$ isotope during CO_2 assimilation by plants. This is detected in the $\delta^{13}C$ signature of root-respired CO_2. The $\delta^{13}C$ signature of SOM becomes more enriched as C derived from roots is metabolised by microbes, leading to a detectable difference in the signature from that from roots. The technique requires the measurement of $\delta^{13}C$ isotopic signatures of the CO_2 respired from the undisturbed ecosystem ($\delta^{13}CR_S$) and those from roots ($\delta^{13}CR_A$) and root-free soil ($\delta^{13}CR_H$).

The proportion of CO_2 emissions derived from heterotrophic respiration, fR_H, is calculated using a two-source mixing model (Equation 2.4) (Millard et al., 2010; Moinet et al., 2016a; Moinet et al., 2016b) where

$$f_{RH} = 1 - (\delta^{13}C_{RS} - \delta^{13}C_{RH})/(\delta^{13}C_{RA} - \delta^{13}C_{RH}). \quad (2.4)$$

The difference in $\delta^{13}C$ values for soil and roots can be further amplified by growing C_4 plants in a soil in which C_3 plants have grown previously since the naturally occurring $\delta^{13}C$ values for C_4 plants (−12 to −15‰,) plants are much more enriched than those for C_3 plants (−25 to −32‰) (Boutton et al., 1999). By growing C_4 or C_3 grassland species in soils, Uchida et al. (2010) showed that R_A as a percentage of the net photosynthesis was (mean ± standard error) 6.4 ± 0.8 and 2.2 ± 0.4% for perennial ryegrass and paspalum (*Paspalum dilatatum* Poir.), respectively

In the absence of plants, the abundance $\delta^{13}C$ stable isotope technique can be used to quantify the priming effect resulting from treatment (Kuzyakov et al., 2000; Shahbaz et al., 2018). For soils treated with added C substrates, priming can be calculated from measurements of $\delta^{13}C$ isotopic signatures from the treated and control soils. The proportion of CO_2 emissions derived from the decomposition of native SOM (fC_{som}) is calculated as described by Kuzyakov and Cheng (2004) from

$$fC_{som} = 1 - ((\delta^{13}C_{sample} - \delta^{13}C_w)/(\delta^{13}C_{substrate} - \delta^{13}C_w)) \quad (2.5)$$

where $\delta^{13}C_w$ the $\delta^{13}C$ value of CO_2 respired from control (with water addition), $\delta^{13}C_{sample}$ is the $\delta^{13}C$ value of CO_2 respired from soils with added labelled C substrates and $\delta^{13}C_{substrate}$ is the $\delta^{13}C$ value of the labelled C substrate.

2.3 Nitrogen cycles and N_2O emissions in irrigated grazed grassland

Human activity emits reactive N (N_r) to the environment. The N_r includes all active N compounds in the atmosphere and biosphere such as ammonia (NH_3), or nitrate (NO_3^-) and N_2O. The goal of managing N cycling in grassland systems is to minimise or prevent N_r losses while maintaining production. Intensively grazed grasslands, often receive both irrigation and fertiliser, and emit more N_2O per unit ground area than arable or forested soils, hence these agricultural soils are important sources of N_2O emissions (Dijkstra et al., 2013; Trost et al., 2013). Studies investigating the effects of irrigation on N_2O emissions have detected high denitrification pulses following fertiliser application, accounting for up to 90% of annual N_2O losses (Trost et al., 2013).

2.3.1 The effects of irrigation on nitrogen cycling in grassland

The net N balance (N_N) in irrigated grassland depends on the relative magnitude of the inputs (N_I) and outputs (N_O) (Equation 2.6) (Bellows, 2001; Mudge et al., 2017). Inputs include fertiliser or effluent (N_F), atmospheric deposition or biological N fixation (N_A), and excreta as urine and dung

(N_D), while outputs include gaseous losses (N_G), harvesting of biomass (N_B), soil runoff, erosion and leaching (N_L) where

$$N_N = N_I - N_O = (N_F + N_A + N_D) - (N_G + N_B + N_L). \quad (2.6)$$

A significant N input in grassland systems is the application of N fertiliser, which is expected to improve soil fertility, and in turn, increases the production, growth, and quality of forage. In New Zealand, the use of N fertiliser has increased > 600% since 1990, from 59,000 t in 1990, to 429,000 t in 2015 (Fertiliser Association of New Zealand, 2018).

Research shows that irrigation potentially increases soil N losses as it directly, and indirectly, increases N leaching to groundwater (Carlton et al., 2018), or gaseous N losses (Owens et al., 2017; Vogeler et al., 2019). Declines in soil N from the organic pool directly impact on the water or air quality, and the lower soil C:N ratios under irrigated grasslands compared with non-irrigated sites are more likely to be N saturated and thus unlikely to retain N (Mudge et al., 2017). Leaching and volatilisation represent significant pathways of N loss in grazed grasslands. However, denitrification is also a major pathway of loss in grazed grasslands used for dairy farming (Friedl et al., 2016).

2.3.2 Pathways for N_2O production in grassland soils

Over a span of 100 years, N_2O is ~300 times more effective than an equal mass of CO_2 at trapping heat and has an average atmospheric lifetime of 114 years (Ciais et al., 2013). Currently, N_2O is also the dominant ozone−depleting substance and it is expected to remain so throughout the 21st century (Ravishankara et al., 2009). In New Zealand, 94% of all N_2O emissions were derived from agricultural soils in 2016, mainly due to urine and dung deposition from grazing animals. Ruminant urine is the major source of N_2O emissions from grazed grassland soils (de Klein et al., 2001). Overall, N_2O emissions have increased by 28% since 1990, and N_2O comprised 22% of all agriculture emissions in 2016 in CO_2 equivalent (CO_2-e) units (Ministry for the Environment, 2018). Direct N_2O emissions from N cycling in agricultural soils predominately derive from microbial processes: nitrification, denitrification, coupled nitrification−denitrification, and nitrifier−denitrification (Fig. 2.4).

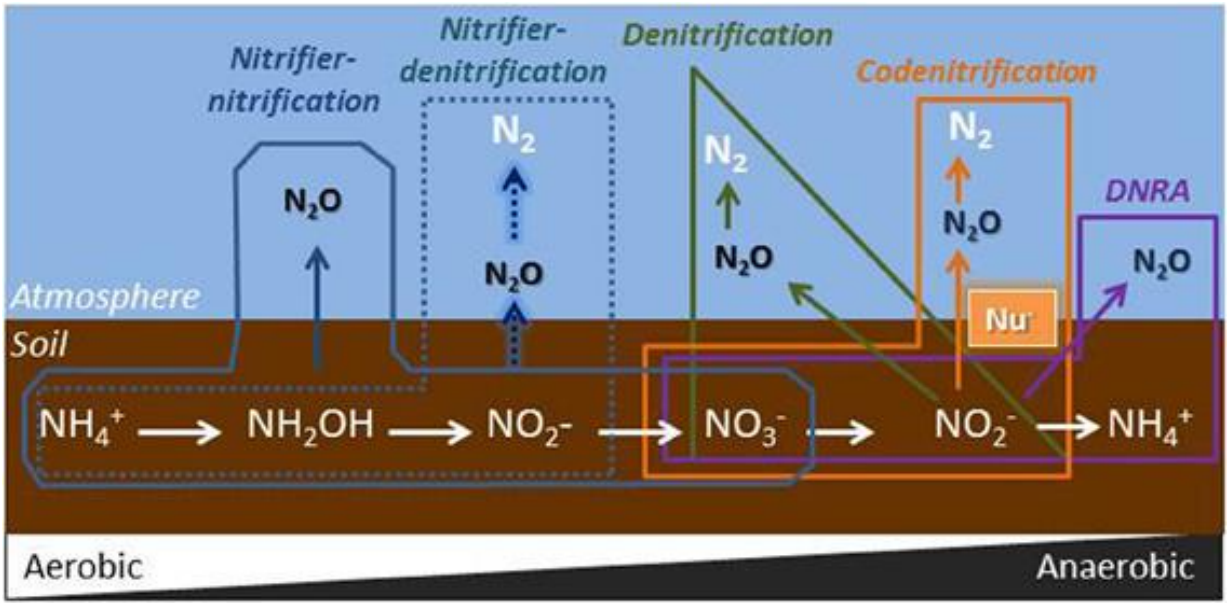

Fig. 2.4 Microbial sources of N_2O during transformations of mineral nitrogen in the soil. Nu^-: nucleophile (e.g., R–NH_2, NH_4^+, amino acids or other organic N compounds). During codenitrification, nitrous acid reacts with a nucleophile in the soil through nitrosation reactions forming a hybrid N–N bond (Spott et al., 2011); DNRA, dissimilatory nitrate reduction to ammonium (Sánchez-García et al., 2014).

2.3.2.1 Nitrification

Nitrification occurs in soils with relatively high O_2 concentrations. It has only recently been confirmed that NH_4^+-N is oxidised, via hydroxylamine (NH_2OH) to nitric oxide (NO), and then nitrite (NO_2^-) (Lundberg et al., 2009) which is then oxidised to nitrate NO_3^- (Sánchez-García et al., 2014) (Fig. 2.4). The NH_2OH may also react with either NO or NO_2^- to form N_2O in the soil. Di et al. (2009) showed that nitrification is driven by bacteria rather than archaea in grassland soils following urine application.

Autotrophic nitrification is the process where nitrifiers gain energy from CO_2 (Wezernak and Gannon, 1967). Heterotrophic nitrification is the process where nitrifiers use organic C for obtaining energy (Robertson and Kuenen, 1990). Heterotrophic nitrification is considered to be common among fungi in soils with a low pH (Kester et al., 1997). A previous study using two acid soils found heterotrophic nitrifiers used organic N compounds for nitrification (Islam et al., 2007).

2.3.2.2 Denitrification

Denitrification is the biological reduction of NO_3^- to N_2. The denitrifiers are predominantly heterotrophic microbes utilising organic C substrates. The microbes involved in denitrification are

facultative anaerobes which can use both O_2 and NO_3^- (or NO_2^-) as the electron acceptors (St John and Hollocher, 1977). Thus, denitrification appears in soils under conditions of O_2 depletion (Russow et al., 2009; Zhu et al., 2013). Nitrous oxide is an obligate intermediate of denitrification (Fig. 2.4) (Russow et al., 2009).

Once NO_3^- is formed, it is usually stable under aerobic conditions. The accumulated NO_3^- can be used by microbes. However, if O_2 becomes limiting, denitrification can produce NO, N_2O or N_2 in the soil. Thus, denitrification, a heterotrophic process, can generally be seen as a microbial response to low O_2 availability in the presence of available NO_3^- and C (St John and Hollocher, 1977; Weier et al., 1993; Bateman and Baggs, 2005). Irrigation contributes to the formation of anaerobic sites in the soil matrix by displacing air from soil pores, creating the anaerobic conditions required for the activity of denitrifying bacteria (Ruser et al., 2006; Friedl et al., 2016; Mumford et al., 2019). In general, a water-filled pore space (WFPS) above 60% favours denitrification with most NO_3^- being denitrified between 70–90% WFPS (Linn and Doran, 1984; Rabot et al., 2015). Soil physical conditions and denitrification are discussed below.

2.3.2.3 Nitrifier-denitrification

Nitrifier−denitrification is dominated by nitrifiers, in which nitrifiers oxidize NH_3 to NO_2^- then reduce the NO_2^- to N_2O and N_2 (Wrage-Mönnig et al., 2018). This likely occurs as soil O_2 becomes limiting (<5%) (Zhu et al., 2013; Wrage-Mönnig et al., 2018). Nitrifier−denitrification can be the predominant source of soil N_2O emissions under certain conditions, high N concentration, low organic C concentration, low O_2 concentration and maybe also low pH (Zhu et al., 2013; Wrage-Mönnig et al., 2018). For example, Wrage-Mönnig et al. (2018) estimated that nitrifiers may account for up to 100% of N_2O emissions from NH_4^+ in soils with the process more significant than that for classical denitrification under some conditions. Recently, using an $\delta^{18}O$ technique, Cardenas et al. (2017) reported the combined contributions of denitrification and nitrifier denitrification to N_2O emission to range from 54–100% and that the highest percentages were due to increases in soil water content.

2.3.2.4 Coupled nitrification-denitrification

Nitrite (NO_2^-) or NO_3^- produced during nitrification can be utilised by denitrifiers. Hence, coupled nitrification−denitrification takes place in soils where both nitrification and denitrification occur concurrently (Wrage-Mönnig et al., 2018). Coupled nitrification−denitrification is a key process of N_2O production. In silty clay loam grassland topsoils in mid-Wales at −5 kPa matric potential, using ^{15}N, nitrification inhibitor, and acetylene, Abbasi and Adams (2000) observed the imbalance between

NO_3^- accumulation and NH_4^+ disappearance, which indicated coupled nitrification-denitrification in the system.

2.3.2.5 Co-denitrification

This process co-occurs with classical denitrification and is thus termed 'co-denitrification'. Co-denitrification results in the formation of N_2O with one N atom originating from the original inorganic-N compound (e.g. NO_2^-) and one N atom from a co-metabolised organic compound (e.g. amino acid, hydroxylamine) (Spott et al., 2011). It was shown by Selbie et al. (2015) that 0.6 t N ha^{-1} was emitted as N_2 by the process of co-denitrification in pastoral soils over 123 days following urine deposition (1 t N ha^{-1}), compared to only 0.01 t N ha^{-1} from denitrification. In addition, using either bacterial, fungal, or combined inhibitors in a laboratory mesocosm experiment where soil received ^{15}N labelled urea, Rex et al. (2018) reported that fungi, not bacteria, dominated total N_2O emissions, and N_2O emissions from co-denitrification.

2.3.2.6 Studying N cycling in grassland and ^{15}N–tracer techniques

The stable isotope of nitrogen ^{15}N has been used as a tracer for the quantification of gross N transformation rates for 60 years (van Groenigen et al., 2015). Since the discovery of ^{15}N (Naudé, 1929), it has been increasingly used as a tracer for studying the environmental impacts of agricultural practices on the N cycle. For example, Clough et al. (2004) examined the effects of soil pH and soil water contents on soil N_2O emissions and using ^{15}N isotope technique to quantify the effects of treatment on soil N_2O emissions. Following urine-N addition, < 0.1 to 1.7% of ^{15}N applied was recovered as N_2O-N over 85 days. The ^{15}N enrichment technique is also useful for partitioning the sources of N_2O emissions.

2.4 Factors influencing CO_2 and N_2O emissions from irrigated grassland

Soil CO_2 emissions from irrigated grassland are affected by the complex interaction of several factors since both R_A and R_H involve chemical, physical and biological processes. The main soil biotic and abiotic factors include, but are not limited to, C substrate availability and quality, soil water content, and O_2 supply (Fig. 2.5) (Linn and Doran, 1984; Luo and Zhou, 2006; Skiba, 2008). Similarly, the processes responsible for the production or consumption of N_2O in soils are strongly affected by the same variables, and N substrate supply (Fig. 2.5) (Tiedje, 1988; Firestone and Davidson, 1989; Skiba, 2008).

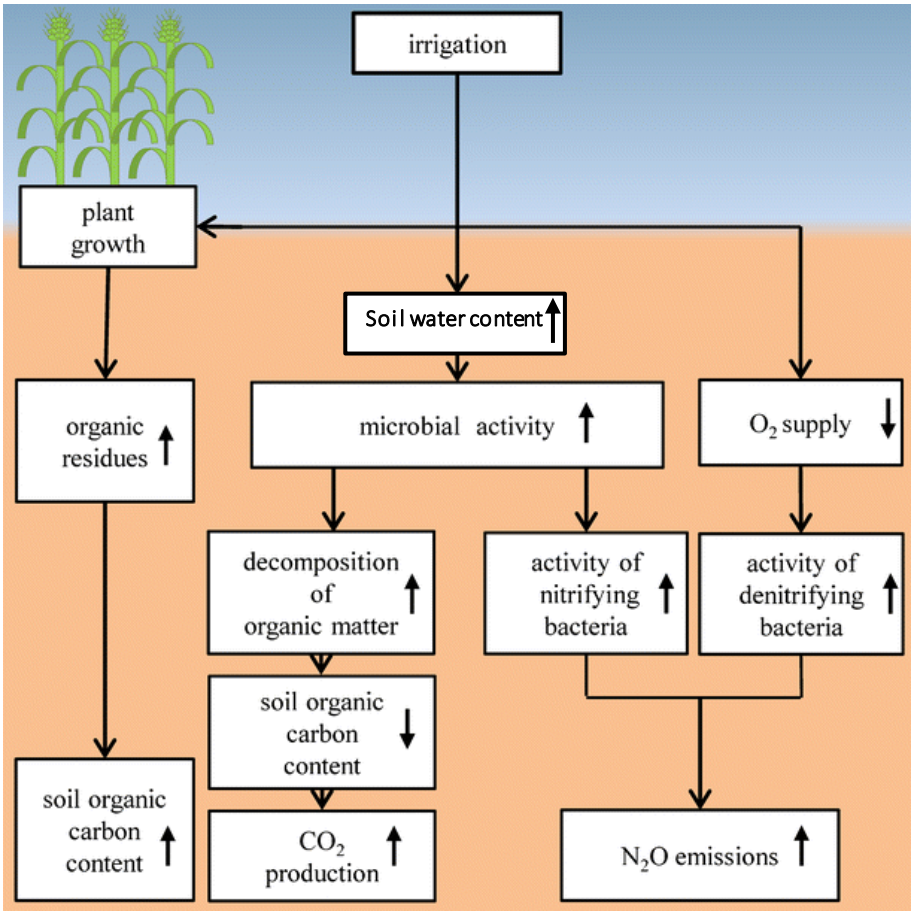

Fig. 2.5 Basic effects of irrigation on soil carbon dioxide (CO_2) and nitrous oxide (N_2O) emissions (increase; up arrow, decrease; down arrow), (Trost et al., 2013).

2.4.1 Soil water content and aeration

The relationship between soil CO_2 emissions and soil water content has been described by linear, logarithmic and quadratic functions (Orchard and Cook, 1983; Linn and Doran, 1984; Davidson et al., 2000). Soil CO_2 emission increases with increasing soil water content until soil pores are filled with water to an extent that O_2 availability becomes limiting (Fig. 2.6). For example, soil CO_2 emissions in an irrigated grassland were consistently greater than that in non-irrigated grassland (Condron et al., 2014). High soil water contents reduce soil CO_2 emission by impeding O_2 diffusion or inhibiting microbial respiration (Fig. 2.6) (Linn and Doran, 1984; Davidson et al., 2000). Soil water content also directly affects the microbial activity and soil CO_2 emission. For example, using continuously labelled plants with depleted ^{13}C in two different soil types, Dijkstra and Cheng (2007) found that on average, a greater priming effect was found in the treatment at higher soil water content (up to 76% increase in soil−derived CO_2−C compared to values for the control) than in the treatment with low soil water content (up to 52% increase). Using data at 34 paired (irrigated and non-irrigated) grassland sites

across New Zealand, Mudge et al. (2017) observed that soil C stocks were lower at irrigated sites compared to those at adjacent non irrigated sites. However, the reason for these results is still mainly uncertain. Thus, studies on the soil CO_2 emissions in irrigated grassland are required to better understand and potentially minimise C losses under irrigation.

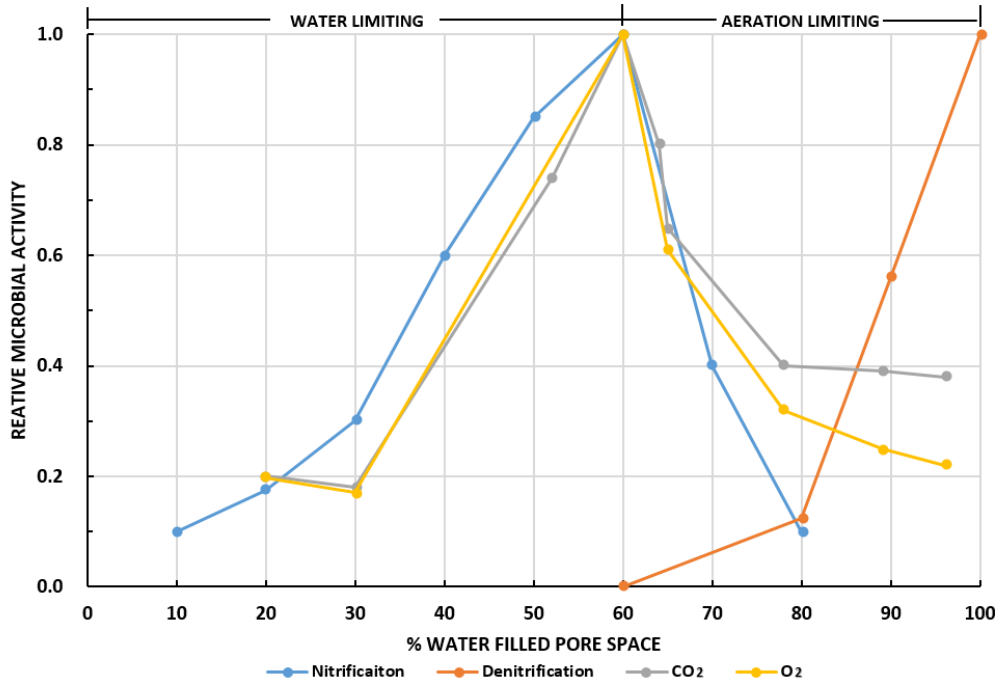

Fig. 2.6 The relationship between water−filled pore space and the relative activity of microbial nitrification, denitrification, and respiration. Adapted from Linn and Doran (1984).

The N_2O:N_2 ratio has also been reported to decrease with increasing soil water content (Davidson, 1992). Water−filled pore space as the main proxy for soil water content has previously been used to relate N_2O emissions with changes in soil water content (Linn and Doran, 1984; Rochette et al., 2004; Barton et al., 2013). N_2O production has been described by a positive linear or exponential increase with WFPS with maximum N_2O emissions occurring at 50−70% WFPS (Flechard et al., 2007). At two poorly drained silt−loam soils in New Zealand, Luo et al. (2008) reported that irrigation increased N_2O emission when soil WFPS increased from 26% to 94%. It was suggested that denitrification dominated N_2O production when WFPS was >60% WFPS in waterlogged soils. With values of WFPS >90%, N_2O emissions decreased as N_2O became reduced to N_2 (Smith et al., 1998). At <60% WFPS, nitrification is considered to dominate the production of N_2O (Bateman and Baggs, 2005). Despite the greater production of N_2O at higher values of WFPS for enhanced denitrification, the release of N_2O from the soil depends on the depth of the source of N_2O production. If the site is an anaerobic microsite in a mostly aerobic soil then the N_2O may diffuse into an oxygenated pore and be emitted from the soil. However, if the N_2O is produced below a saturated zone it may be reduced to N_2

before being released (Smith et al., 2003; Klefoth et al., 2014). Moreover, a nearly complete filling of the WFPS over a long period of time may lead to a decrease in N_2O emissions since, under strict anaerobic conditions, N_2O is completely reduced to N_2 (Huang et al., 2007; Klefoth et al., 2014). Furthermore, WFPS cannot express the physical force with which water is held in soil, soil pore connectivity or tortuosity (Linn and Doran, 1984; Farquharson and Baldock, 2008), which are crucial in regulating soil gas transport. Gas diffusion in the soil is a function of both soil porosity and soil water content (Millington, 1959; Davidson and Trumbore, 1995).

Soil diffusivity is a variable that can be used to describe both O_2 availability and CO_2 and N_2O transfer from the soil to the atmosphere (Davidson and Trumbore, 1995). Soil relative gas diffusivity (D_P/D_O) has been used to describe the interactive effects of soil water content and soil bulk density (ρ_b) on soil tortuosity and was shown to be a better predictor of soil aeration status (Balaine et al., 2013; Smith, 2017; Chamindu Deepagoda et al., 2019). D_P/D_O is defined as the ratio of the soil−gas diffusion coefficient (D_P) to the free−air gas diffusion coefficient (D_O). D_P/D_O has been shown to be a key variable able to predict co-denitrification for N_2O and N_2 emissions under variable soil water content and ρ_b (Rolston and Moldrup, 2002; Balaine et al., 2013; Harrison-Kirk et al., 2015; Balaine et al., 2016; Owens et al., 2016). For example, following NO_3^- addition to repacked soil cores on tension tables, Balaine et al. (2013) observed that maximum N_2O emissions occurred at a value of D_P/D_O of 0.006 that was independent of soil bulk density. However, no such general relationship could be established with WFPS (Fig. 2.7). Moreover, using repacked soil cores and ^{15}N−labeled synthetic urine with soil on tension tables, Harrison-Kirk et al. (2015) also observed that the relationship between $N_2O + N_2$ emissions and D_P/D_O was stronger than for N_2O emissions. Balaine et al. (2016) also found that D_P/D_O regulated both N_2O and N_2 emissions. However, there is a lack of such studies relating soil CO_2 emissions or both CO_2 and N_2O emissions to D_P/D_O. Additionally, irrigation management directly affects soil water content and thereby WFPS and D_P/D_O, while many studies have established relationships between the intensity of irrigation and N_2O emissions (Scheer et al., 2008; Owens et al., 2016; Carlton et al., 2018; Mumford et al., 2019), there has been less emphasis on the effects of the frequency of water application (Vogeler et al., 2019).

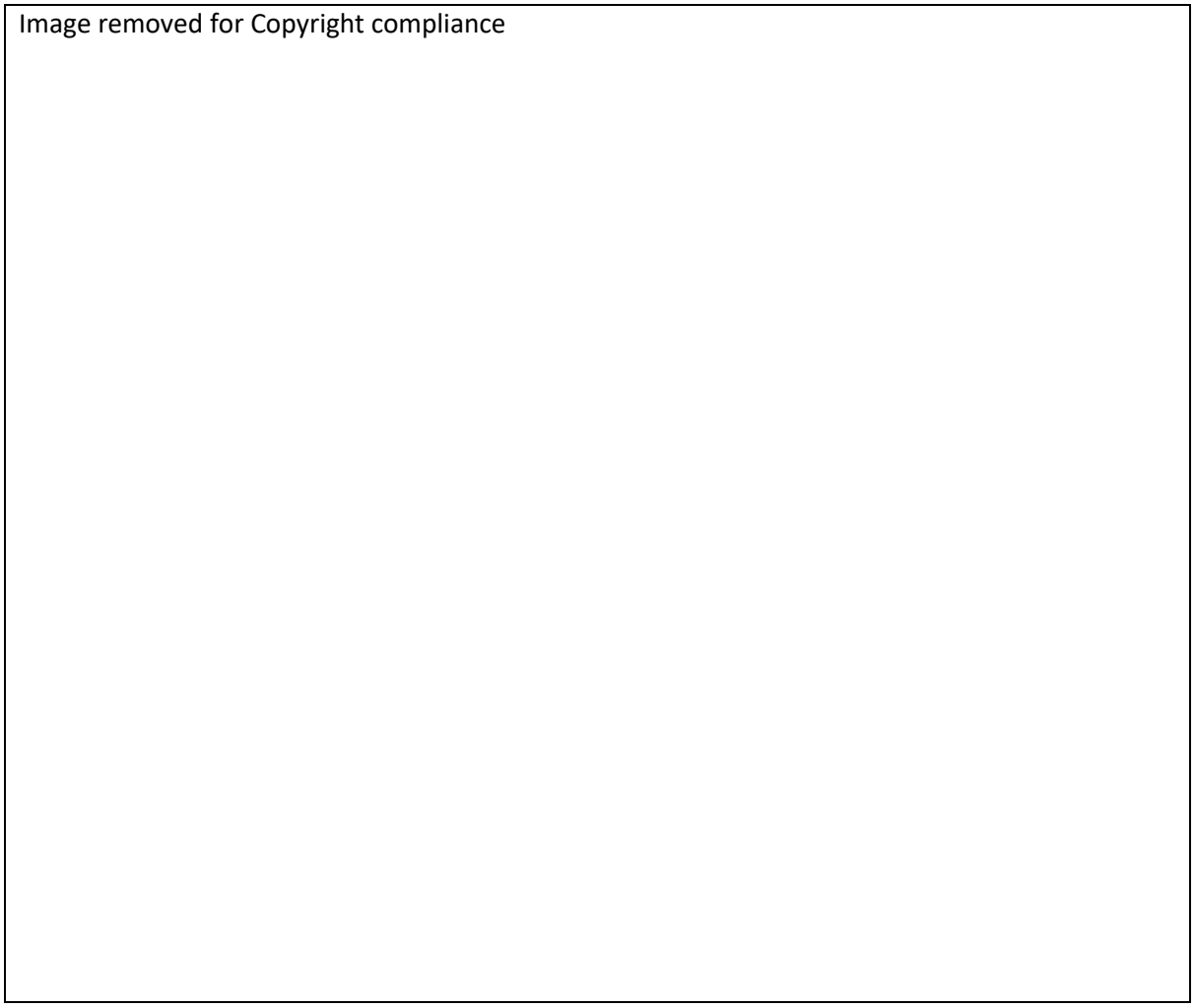

Fig. 2.7 Relationship of N_2O emissions with (a) water–filled pore space (%) (b) relative soil gas diffusivity (D_P/D_O) at varying soil bulk density (Mg m^{-3}) (Balaine et al., 2013).

2.4.2 Soil carbon and nitrogen

Soil respiration is resourced mainly from root-derived C that is influenced by photosynthesis as it exudes from or is sloughed off plant roots. In a one year clipping and shading experiment in a tallgrass prairie of the Great Plains, United States, Wan and Luo (2003) showed that annual average values of R_S in clipped, shaded, and clipped plus shaded plots were 34, 31, and 48% lower than that those in the control plots, respectively. In addition, drying and rewetting resulting from irrigation can have a major effect on soil C availability. For example, water deficits reduced soil microbial activity while rewetting increased microbial activity and led to a pulse of R_S (Bottner, 1985). Using soils from three contrasting ecosystems, Eberwein et al. (2015) demonstrated that the increase in the half-saturation of WFPS led to a decrease in R_S when C was limited, and both C–N and C-temperature

interactions were markedly similar between sites. This highlighted the importance of C availability for the regulation of R_S.

Organic matter such as plant litter, root exudates, or native SOM provides C and energy for heterotrophic denitrifying organisms (Skiba, 2008). Studies have found that the N_2O product ratio of denitrification, defined as $N_2O:(N_2O+N_2)$, in soils may be affected by the relative availability of organic C and NO_3^- (Miller et al., 2008) and the rate of C substrate supplied (Baggs et al., 2000). It is generally considered that increasing C availability decreases the ratio of $N_2O:N_2$ (Weier et al., 1993; Morley et al., 2014). However, the effects of C availability on the absolute and relative amounts of N_2O and N_2 production also vary with NO_3^- concentration (Miller et al., 2008), and of the availability of C substrates (Morley and Baggs, 2010; Morley et al., 2014). Relative to the effects of added sugars, organic acids can be metabolically converted for entry into the tricarboxylic acid cycle (Gunina et al., 2014), and can promote dissolution of protective mineral phases (Keiluweit et al., 2015). For instance, Morley et al. (2014) has reported that the organic acid, acetate, was more efficient in enhancing N_2O reduction relative to glucose. Normally, NO_3^- inhibits the rate of N_2O reduction to N_2 producing a higher $N_2O:(N_2O+N_2)$ ratio under similar water content and O_2 conditions (Firestone and Davidson, 1989). The change in the $N_2O:N_2$ ratio from denitrification that results from changing the labile $C:NO_3^-$ ratio can be explained by changes in enzyme status, and/or the diffusion rate of NO_3^- into denitrifying microsites (Weymann et al., 2010).

Additionally, C compounds released as exudates by plants can also affect two major soil N processes: nitrification and denitrification (Coskun et al., 2017). For example, root exudate−C increases potential heterotrophic microbial immobilisation and has been shown to be effective in decreasing the risk of N loss (Fisk et al., 2015). For instance, applying glucose, glutamine or citric acid and KNO_3 to a sandy loam soil, Giles et al. (2017) indicated that differences in N_2 and N_2O emissions were not caused by selection for denitrifiers but likely driven by differences in substrate use efficiency and subsequent differences in C partitioning between growth and respiration. In an open chemostat culture, enriched from activated sludge under strict anoxia, van den Berg et al. (2017) found that the nature of the electron donor influenced the outcome of competition between denitrification and dissimilatory reduction to ammonium and denitrification: fermentative conversions have an influence on the type of C source available for nitrate reduction and thus potentially affect the relative occurrence of denitrification. The role of organic C in regulating N_2O production has been investigated by Morley et al. (2014), who explained the effects of addition of a range of C compounds (amino acids, organic acids, and sugars) on denitrifier $N_2:N_2O$ ratio. The authors concluded that a soil's ability to reduce N_2O to N_2 is C substrate-dependent. However, only one soil was used and with one level of soil water content, so further studies are required to better

understand the effects of C substrate type and O_2 supply on N_2O, N_2 and CO_2 emissions. Further, there remains a need to determine the effects of C substrate additions on both priming effects of C substrates and the relative contribution of these sources to N_2O emissions.

The addition of N affects a range of biogeochemical processes that regulate the production and consumption of CO_2 (Templer et al., 2012). Nitrogen additions enhance rates of R_H and R_A because increased soil N availability affects labile SOC concentration and root biomass (Verburg et al., 2004). In Ireland, losses of up to 29 kg N_2O–N ha^{-1} yr^{-1} have been recorded from grassland with an N fertiliser application rate of 390 kg N ha^{-1} yr^{-1} (Hyde et al., 2006). Results, from 22 investigations on the impact of irrigation on soil C concentrations and N_2O emissions indicated that average N_2O emissions increased 87% under irrigation in combination with addition of N fertiliser, while the addition of fertilisers only increased N_2O emissions by 7% (Trost et al., 2013). In most cases, the availability of a reactive N compound is important for N_2O emission. However, few studies have concurrently measured both CO_2 and N_2O emissions from soil with added C substrate in the presence of N substrate (Giles et al., 2017).

2.4.3 Soil pH

Soil pH affects soil SOM decomposition rate and N_2O emission. Soil pH can directly regulate the activities of microbes and enzymes (Jones et al., 2019). Feng et al. (2018) found annual R_S in Chinese grasslands, where soil pH ranged from neutral to alkaline soils, was correlated with soil pH. Soil pH has significant effects on the priming of SOM. Adding organic acids to the soil using an artificial root system, Keiluweit et al. (2015) observed that reduced SOM decomposition in the presence of acetate was attributable to an increase in soil pH and that oxalate increased SOM decomposition while reducing soil pH. In addition, using a ^{14}C–labelling approach across 970 agricultural soils, Jones et al. (2019) showed that maintaining soil pH above 5.5 promoted greater microbial C use efficiency.

Soil pH also has critical effects on N_2O emissions as it influences rates of denitrification and the N_2O:N_2 ratio (Firestone and Davidson, 1989; ŠImek and Cooper, 2002). In pure culture or natural systems, denitrification rate is positively related to pH (Knowles, 1982). Recently, using the combined approaches of ^{15}N and ^{18}O labelling with transcriptome analyses, Duan et al. (2019) found that nitrification dominated N_2O emission in alkaline soils, and heterotrophic denitrification was the main source of N_2O in acidic soils. However, Šimek et al. (2002) suggested that denitrifying enzyme activity had no relation to soil pH because soil denitrifiers could adapt to various values of soil pH. Čuhel et al. (2010) found that the N_2O:(N_2O+N_2) ratio increased with decreasing soil pH due to changes in total denitrification activity, while no changes in N_2O production were observed in a

grazed grassland over 10 months. In general, an increase in soil pH enhances nitrification with the optimum pH from 3.0 to 9.5 depending on the site-specific conditions (Sahrawat, 2008). The effects of soil pH on N_2O emissions also depend on soil water content. Applying synthetic bovine urine to repacked soil cores, limed to varying soil pH levels, Clough et al. (2004) found that under saturated soil conditions, the cumulative N_2O-N emissions increased with soil pH between pH 4.7 to 6.6. In the field capacity treatment, the cumulative N_2O-N emission decreased with increasing soil pH.

At low pH values, N_2O reductase is inhibited, such that the overall rate of denitrification decreases, but the mole fraction of N_2O produced increases (Knowles, 1982). A common feature of strains of denitrifying bacteria tested in a laboratory study was that the N_2O:(N_2+N_2O) ratio was correlated with acidity, apparently attributable to interference with the assembly of the enzyme N_2O reductase (Bakken et al., 2012). Numerous laboratory and field studies have demonstrated that the ratio N_2O:N_2 is increased when the pH of soils is reduced (Šimek et al., 2002). In a review exploring the biotic transformations of nitrogenous compounds that occur during denitrification in temperate grasslands, Saggar et al. (2013) also found that decreasing soil pH led to increased N_2O:N_2 ratios.

2.5 Objective and Hypotheses

Based on the literature it is clear that the interactive effects of irrigation and substrate supply (C and N) regulate soil respiration and denitrification and thus the losses of CO_2, N_2O, and N_2. Irrigation is used to promote plant growth. Subsequently, C compounds released by plant roots will affect soil gas diffusion as the change in soil water content, thereby affecting soil CO_2 and N_2O formation mechanisms (Fig. 2.8). Carbon substrate type and water content have been shown to affect N_2O:N_2 ratios, but this has not been examined in multiple soils with associated CO_2 losses. Thus it is hypothesised that:

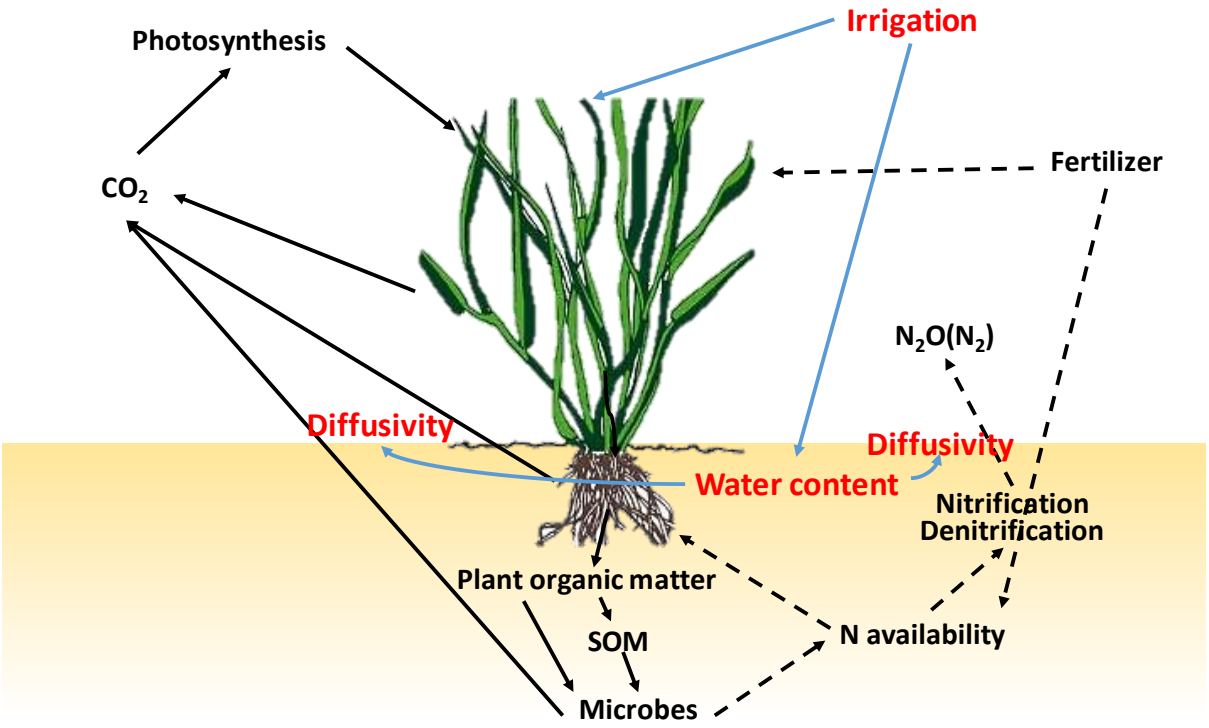

Fig. 2.8 Conceptual diagram depicting the interactive responses of plant and soil carbon, nitrogen and water in response to irrigation and addition of nitrogen fertiliser.

i. Increasing soil water content will increase the rate of denitrification while decreasing the $N_2O:(N_2O+N_2)$ ratio regardless of soil or substrate type. As previously shown, in one soil, the effect of acetate in reducing the $N_2O:(N_2O+N_2)$ ratio will be consistent with soil types. (Chapter 3).

ii. Positive priming by soil C substrate will increase N availability from SOM and thus lead to increased SOM derived N_2O emissions, with organic acids generating greater SOM priming than glucose. (Chapter 4).

iii. Increasing cumulative soil water deficit, resulting from decreasing irrigation frequency, will decrease net ecosystem CO_2 exchange due to reduced plant respiration, and an increased ratio of root to heterotrophic respiration, resulting in a decreased soil N_2O emissions due to increased soil aerobic conditions. (Chapter 5).

Chapter 3 Emissions of nitrous oxide, dinitrogen and carbon dioxide from three soils amended with carbon substrates under varying soil matric potential

3.1 Abstract

Existing Carbon (C) substrate is critical for regulating denitrification, a process that results in nitrous oxide (N_2O) and dinitrogen (N_2) emissions from soil. However, the impacts of C substrates on soil carbon dioxide (CO_2) and N_2O emissions, under varying soil types and soil water contents, are not well studied. Three repacked Pallic grassland soils containing NO_3^--^{15}N were held at three levels of matric potential (ψ, −3, −5 and −7 kPa), while receiving daily substrate additions (acetate, glucose, water control) for 14 days. The CO_2 and N_2O emissions were monitored daily. Additionally, the N_2O:(N_2+N_2O) ratios were determined using ^{15}N methods on days 3 and 14. Results showed that across all soils, N_2O peak emissions were higher for soils treated with glucose, with a range (± SD) of 0.1 ± 0.0 to 42.7 ± 2.1 mg N m^{-2} h^{-1}. The highest cumulative N_2O emission (2.5 ± 0.2 g N m^{-2}) was measured in glucose-treated soil at a ψ of −3 kPa. On day 14, acetate resulted in 2-fold higher N_2 emissions compared to glucose in soils at low diffusivities. The N_2O:(N_2O+N_2) emissions ratios varied with soil type (0.91-0.80) on day 3. Cumulative CO_2 emissions increased with increasing soil diffusivity and soils amended with glucose had higher cumulative CO_2-C emissions, ranging from 22.5 ± 1.3 to 36.6 ± 1.8, g C m^{-2}. Collectively, the increase of N_2O, N_2 and CO_2 emissions in response to acetate or glucose addition depended on both soil and soil matric potential. The findings indicate that non-fermentable substrates enhance denitrification.

Keywords: acetate; glucose; greenhouse gas emissions; matric potential; soil diffusivity

3.2 Introduction

Nitrous oxide (N_2O) is a potent greenhouse gas and N_2O emissions from agricultural sources and synthetic fertilisers (Davidson, 2009) account for 6% of total anthropogenic radiative forcing (Davidson, 2009). The primary processes responsible for generating N_2O in terrestrial ecosystems are nitrification and denitrification. Nitrification occurs under aerobic conditions and is performed by chemolithotrophic soil microorganisms, ammonia oxidising bacteria (AOB) and archaea (AOA), that ultimately convert ammonia, via nitrite (NO_2^-) to nitrate (NO_3^-) (Firestone and Davidson, 1989). If

conditions become hypoxic, AOB perform a process known as 'nitrifier denitrification' which results in N_2O production as a result of NO_2^- reduction, while under anaerobic conditions AOB may also produce N_2O via the anaerobic oxidation of hydroxylamine (Stein, 2019). In addition, the nitrification intermediaries (hydroxylamine, nitric oxide (NO), NO_2^-) may undergo abiotic or biotic processes to produce N_2O (Stein, 2019). Denitrification is classically defined as the sequential reduction of NO_3^- to dinitrogen (N_2), through the obligate intermediaries of NO_2^-, NO, and N_2O, which occurs under anaerobic conditions (Zumft, 1997). However, the individual steps within the denitrification process may also occur concurrently (Liu et al., 2013) within a single taxa or divided across multiple taxa (Hallin et al., 2018). Most denitrifiers are aerobic heterotrophs that use a carbon (C) source as an electron donor to reduce an N oxide under anaerobic conditions (Zumft, 1997). In grassland soils, C sources include the mineralisation of SOM, plant exudates, manures and slurries (Laughlin and Stevens, 2002; Henry et al., 2008).

Another microbial pathway for N_2 production is anaerobic ammonia oxidation (anammox) but its role in N_2 production in grassland soils is unknown with prior work identifying paddy and peat soils as important for this process (Hu et al., 2011). Microbial production of N_2O and the potential for reduction of N_2O to environmentally benign N_2 gas is thus dependent on the soil's oxygen (O_2) status, and supply of substrates (N and C). Soil O_2 status depends strongly on soil matric potential with an associated increase in soil water content from field capacity (-10 kPa) to near saturation (-0.2 kPa) shown to reduce soil O_2 supply, as a result of a decrease in soil gas diffusivity (Balaine et al., 2016). Owens et al. (2017) confirmed, *in situ*, a strong relationship between soil gas diffusivity and N_2O emissions following urea application to grassland soil. Nitrous oxide reductase is particularly sensitive to O_2 concentration and Balaine et al. (2016) showed that the ratio of soil N_2O:N_2 emissions declined with increasing soil water content as a result of the decrease in soil gas diffusivity increasing anaerobic conditions. The quantity and quality of soil C also affect the rate of denitrification and the N_2O:N_2 ratio ((Firestone and Davidson, 1989; Gillam et al., 2008; Senbayram et al., 2012). As the quantity of C available to denitrifiers increases, the rate of denitrification increases if sufficient NO_3^- substrate and anaerobic conditions are present (Senbayram et al., 2012).

Dual regulation of N_2O production and reduction by C and O_2 was demonstrated by Morley and Baggs (2010) where C quality interacted with the initial O_2 concentration of the headspace above soil slurries: butyrate and glutamic acid addition caused greater N_2O production compared to the effects of glucose and mannitol after 110 hours in the presence of NO_3^- at 21% O_2 but not at ~2% O_2. Using repacked sandy loam soil cores maintained at 80% water-filled pore space. Morley et al. (2014) further examined the relative effects of organic acids and sugars on the N_2O:N_2 ratio, over 14 days in

the presence of NO_3^-. The authors found that the reduction of N_2O to N_2 was enhanced (112 – 186%) under organic acids when compared with the effects of glucose. It was suggested that these responses may differ with different soil types (Morley et al., 2014). However, since the studies of Morley and Baggs (2010) and Morley et al. (2014), there appear to be no other studies examining the effects of C substrate type and O_2 supply, on soil type, with respect to N_2O, N_2 and CO_2 emissions.

The objectives of this study were to assess the effects of adding daily inputs of C, either glucose or acetate, on N_2O, N_2, and CO_2 production over 14 days in repacked cores. We extended the earlier work of Morley et al. (2014) to compare the findings for three soil types held at three values of soil matric potential. We hypothesised that (i) increasing soil matric potential would increase the rate of denitrification but decrease the N_2O:(N_2O+N_2) ratio regardless of soil or substrate type due to reduced soil gas diffusivity since low O_2 supply promotes N_2 production (Morley and Baggs, 2010; Balaine et al., 2016), (ii) acetate substrate would enhance N_2O reduction relative to glucose, and that this would occur regardless of soil type, and (iii) CO_2 emissions from C substrates would not differ between soil type.

3.3 Materials and methods

3.3.1 Experimental design

Soils were sampled (0-150 mm depth) from three grazed grassland sites all dominated by perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.). The sites were located within a 5 km distance with the same climatic conditions but with different soil types. The soils were collected from the Ashley Dene dairy farm (AD, latitude 43° 65’ S, longitude 172° 35’ E, elevation above sea level 34 m, Mottled Argillic Pallic Soil (Hewitt, 2010), Udic Ustochrept (Soil Survey Staff, 2014), the Lincoln University long-term dairy farm (LU, 43° 65’ S,172° 48’ E, Typic Immature Pallic soil (Hewitt, 2010), Typic Haplustept (Soil Survey Staff, 2014), and the Lincoln University demonstration farm (LD, 43° 65’ S,172° 44’ E, Typic Immature Pallic soil (Hewitt, 2010), Typic Haplustept (Soil Survey Staff, 2014). The soils were brought to the laboratory, air-dried and then sieved (≤ 2 mm; Fig. A1), with any visible plant material removed, and stored at 4°C. Soil total C and total nitrogen (N) concentrations were determined by subsampling the soil, and analysing it on an Elementar Vario-Max CN Elemental Analyser (Elementar GmbH, Hanau, Germany) (Table 3.1). Texture analyses were performed using a laser diffraction particle analyser (Mastersizer 3000, Malvern Panalytical, U.K.). Soil pH was measured on deionised water extracts (Rowell, 2014). Sieved soil was packed into stainless steel rings (73 mm internal diameter, 74 mm depth) to a depth of 41 mm (Fig. A2), to achieve a soil bulk

density (ρ_b) of 1.1 Mg m^{-3}. The bottom of each soil core was covered with a fine nylon mesh (25 µm) to prevent any soil loss. Water holding capacity of each soil was determined by immersing the soil cores in water for 2 hours and then draining for 24 hours (Priha and Smolander, 1999).

The factorial experiment consisted of four replicates of three factors: soil type, matric potential and C substrate; comprising three levels each of soil type (AD, LU, LD), soil matric potential (ψ; −3, −5 and −7 kPa), and C substrate (acetate, glucose, or water as a control). Glucose was selected because it is used commonly as a C source for SOM priming (Kuzyakov et al., 2000) and to determine C substrate limitation when determining soil denitrification potential (Morley et al., 2014). Acetate, applied as sodium acetate, was selected because its effect on N_2O production from denitrification has been shown to differ from that of glucose (Morley et al., 2014). Soil ψ levels were based on those previously observed to give a range of denitrification rates (Balaine et al., 2016). In total, 216 soil cores were packed and this allowed for the destructive analyses of a fully replicated set of treatments on day 3 of the experiment and at the end of the experiment on day 14 and aligned with the ^{15}N gas emission sampling undertaken on days 3 and 14 as described below.

Table 3.1 Soil physical and chemical properties for the soils at Ashley Dene dairy farm (AD), Lincoln University dairy farm (LU), and Lincoln University Demonstration Farm (LD). Data shown are mean ± SD, n=3. Significance levels are given for differences between sites ($P < 0.05$) and means denoted by a different letter.

Site	Total carbon (g kg^{-1})	Total nitrogen (g kg^{-1})	Clay % (< 2 µm)	Silt % (2−63 µm)	Sand % (> 63 µm)	Water holding capacity (g H_2O g $soil^{-1}$)	pH
AD	32.3±0.4 b	3.3±0.0 b	12	46	42	0.39±0.02	6.2 ± 0.3 a
LU	46.6±1.0 a	4.5±0.2 a	16	48	36	0.55±0.01	6.0 ± 0.1 a
LD	45.3±1.7 a	4.8±0.2 a	17	46	37	0.55±0.01	5.8 ± 0.2 a

Soils were maintained at the set soil ψ values by placing the cores on tension tables (Fig. A4) after they had been saturated with distilled water and allowed to drain for 4 days (Romano et al., 2002). Then 1 mL of a KNO_3, ^{15}N enriched, solution (300 µg N g^{-1} soil or 27.6 mg N mL^{-1}; 40 atom% excess ^{15}N, Cambridge Isotope Laboratories Inc., USA) was applied. The day of KNO_3 addition was defined as

day 1 of the experiment. Subsequently, a total of 0.9 mL of C solution was added daily for 14 days (80 μg C g^{-1} soil or 16.4 mg C mL^{-1}) by injecting 0.18 mL of the C solution at 5 evenly spaced points, to a depth of 20 mm, using a syringe. Tension tables and soil cores were maintained at an average temperature of 20°C.

3.3.2 Soil analyses

Soil surface pH was measured with a flat surface pH meter (Broadley James Corp., Irvine, California) prior to destructive sampling. Soil cores extruded from the stainless steel rings were homogenised manually and subsampled to determine gravimetric water content (θ_g) by drying at 105°C for 24 hours. Water-filled pore space (WFPS) was calculated using θ_g, ρ_b and, for all soils, an assumed particle density of 2.65 Mg m^{-3} (Nimmo, 2004). Dissolved organic carbon (DOC) concentrations were determined after extracting soil samples with deionised water for 1 hour and then centrifuging (3500 rpm) the extracts for 20 min before filtering through 0.45 μm cellulose nitrate membrane filters (Ghani et al., 2003). The DOC concentrations were determined on a Shimadzu TOC analyser (Shimadzu Oceania Ltd., Sydney, Australia). Soil inorganic-N was determined by extracting soil subsamples with 2 M KCl for 1 hour (1:10 ratio of soil:KCl), centrifuging (3500 rpm) and filtering (Whatman grade 42 paper). The NO_3^--N and NH_4^+-N concentrations of the KCl extracts were determined using flow injection analysis (Blakemore et al., 1987).

3.3.3 Emissions of N_2O, N_2 and CO_2, and measurement of relative gas diffusivity

Daily measurements of emissions were made by placing soil cores into glass jars (1 L) equipped with a gas-tight lid fitted with a rubber septa (Fig. A7). A syringe fitted with a two-way stopcock and a 25G hypodermic needle was used to remove gas samples (10 mL) for measurement of N_2O concentrations, at 30 and 60 minutes after the jar was sealed. These samples were injected into previously evacuated 6 mL Exetainer® vials (Labco Ltd., High Wycombe, UK) for analysis on a gas chromatograph (SRI-8610, Torrance, CA) equipped with a ^{63}Ni electron capture detector. Increases in N_2O concentration over time (0, 30, and 60 min) were used to calculate rates of N_2O emissions according to Hutchinson and Mosier (1981). Additional gas samples (15 mL) were taken on days 3 and 14, after 180 min, for determination of the ^{15}N enrichment of the N_2O and N_2 evolved using the ^{15}N gas-flux method (Mulvaney and Boast, 1986). These samples were injected into pre-evacuated 12 mL Exetainer® vials. A continuous flow isotope ratio mass spectrometer (CFIRMS, Sercon 20-22; Sercon, Chesire, U.K) interfaced to a TGII cryofocusing unit (Sercon, Chesire, U.K) was used to measure the ion currents 44, 45, and 46 for N_2O, and 28, 29 and 30 for N_2. Ion currents were

subsequently used to determine the N_2O-^{15}N enrichment (Stevens et al., 1998) and for calculating the N_2 emissions (μg N m^{-2} h^{-1}) (Mulvaney and Boast, 1986). Days 3 and 14 were selected for determining the N_2O and N_2 emissions since, at day 3 it was expected that N_2O emissions would be approximately near their peak, while at day 14 it was expected that the soil emissions would be representative of steady state.

Soil CO_2 emissions (μmol m^{-2} s^{-1}) were measured by placing a static chamber on top of the soil core that was connected to an automatic soil respiration system (Model LI-8100, Li-COR Inc., Lincoln, Nebraska, USA) (Fig. A5).

For both CO_2 and N_2O, daily emissions were calculated and integrated over time to give cumulative emissions over 14 days. In the absence of measurements on days 8, 10, 12 and 13, when soil CO_2 emissions have reached steady state, and soil N_2O emissions have dramatically declined, the Loess model (Cleveland and Devlin, 1988) was used to estimate gas emissions.

Soil relative gas diffusivity (D_p/D_o) was measured using a gas diffusion chamber (Fig. A9) (Balaine et al., 2013), which was engineered following Rolston and Moldrup (2002). Briefly, a chamber containing a calibrated oxygen (O_2) sensor (KE-25, Figaro Engineering Inc., Osaka, Japan) was purged with O_2-free air (90% Ar and 10% N_2) while the base of the soil core was isolated from the chamber. Once the chamber O_2 concentration fell to zero, the base of the soil core was exposed to the O_2-free chamber atmosphere and the concentrations of O_2 for the gas diffusing through the soil core into the chamber was measured after 120 to 180 min. The technique assumes that any error in the calculated value of D_p (O_2 diffusion coefficient in soil), due to O_2 consumption was negligible (Moldrup et al., 2000). D_p was calculated from the rate of O_2 increase in the chamber using regression analysis (Rolston and Moldrup, 2002). All diffusivity measurements were made at 20°C and the value of D_o at this temperature was 0.072 m^2 h^{-1} (Currie, 1960).

3.3.4 Data analyses

The effects of the treatments on soil CO_2 emissions were tested for significance using a non-linear mixed-effect (NLME) model using the 'nlme' package of R (Pinheiro et al., 2014). Each CO_2 emission measurement was treated as a sample, with soil type, soil ψ, and substrates set as random effect factors. To account for non-independence of repeated measurements, the replicate number was included as a random effect in each model. A three parameter rectangular hyperbola (Crawley, 2007) was fitted to the data where

$$R_s = a - b \times e^{-c \times t} \quad (3.1)$$

where, R_s is the CO_2 emission rate; t is time; a is the value for steady-state CO_2 emissions; b is the difference between the value of CO_2 emissions on a given day and the value of CO_2 emissions on day 0, and the parameter c describes the shape of the curve. Model comparisons were based on Akaike's Information Criterion (AIC). The model with the lowest AIC indicated the best-fitting model (Anderson and Burnham, 2002) and analyses of residuals were undertaken to check the model assumptions. Parameter values were compared using Tukey's *HSD* test in the 'agricolae' package of R (De Mendiburu, 2014).

The effects of soil type, C substrate, and soil ψ, and their interactions on soil pH, DOC, NO_3^--N, NH_4^+-N concentrations, the N_2O:(N_2+N_2O) ratio, and cumulative values of CO_2-C emissions and N_2O-N emissions were tested using an ANOVA in the 'agricolae' package of R version 1.3.1 (De Mendiburu, 2014). In addition, cumulative values of CO_2-C emissions and N_2O-N emissions were compared using Tukey's *HSD* test in the 'agricolae' package of R (De Mendiburu, 2014).

3.4 Results

3.4.1 Soil physical and chemical properties

Soil surface pH increased with either acetate or glucose addition compared to the water treatment, regardless of soil type. On day 3 soil pH values under the acetate treatment (range, 6.3-7.2) were higher than those under glucose (5.9-6.5), which in turn were higher than those in the control (5.4-5.7), (P<0.001, Table 3.2). Similar findings were observed on day 14 with soil surface pH values under the acetate, glucose and water treatments ranging from 8.7-8.8, 7.1-8.3, and 5.3-6.0, respectively (Table 3.3). There was no effect of soil ψ or soil type on the soil surface pH on either day 3 or 14.

As expected, on both days 3 and 14, soil water content declined in all soils (P<0.001) as soil ψ decreased (−3 to −7 kPa): values of WFPS in the AD, LU, and LD soils ranged from 71 to 55%, 90 to 83%, and 94 to 90%, respectively. For the LD soil, WFPS declined as soil ψ decreased from −3 kPa (94%) to −5 kPa (90%) but not from −5 to −7 kPa. When averaged across all soil ψ treatments, soil water content was higher (P<0.001) for the LU and LD soils than that for the AD soil. There was no effect of C substrate addition on soil water content. Average relative soil gas diffusivity (D_p/D_o) in the AD soil was 0.0040, 0.0110, and 0.0154 at −3, −5 and −7 kPa, respectively, while for the LU and LD soils D_p/D_o was <0.006 regardless of soil matric potential (range 0.0026-0.0058; Table S3.1).

The AD soil contained less organic C than the LU and LD soils (P<0.05) and, as a consequence of its higher sand content (Table 3.1) held less water at field capacity. No such differences occurred between the LU and LD soil.

On day 3, DOC concentrations in the acetate (66-254 μg C g^{-1} soil) and glucose (50-254 μg C g^{-1} soil) treatments were higher than those under the control treatment (40-105 μg C g^{-1} soil) in both the AD and LU soils (P<0.05, Table 3.2). For the LD soil on day 3, the DOC concentrations in the acetate treatment (183-289 μg C g^{-1} soil) were higher than those for the control treatment (P<0.05) but the glucose treatment DOC concentrations were not (138-244 μg C g^{-1} soil; Table 3.2). On day 14, for all soils, the DOC concentrations in the acetate (180-789 μg C g^{-1} soil) and glucose (68-520 μg C g^{-1} soil) treatments were, when averaged across soil ψ treatments, higher (P<0.05) than those in the control treatment (24-188 μg C g^{-1} soil; Table 3.3). Soil type influenced DOC concentrations on day 14: for both LU and LD soils, the DOC concentrations were higher than those in the AD soil for acetate, glucose and the control treatments at all levels of soil ψ (P<0.001; Table 3.3).

On day 3 soil NO_3^--N concentrations were unaffected by treatments with values ranging from 218-361 μg NO_3^--N g^{-1} soil (Table 3.2). On day 14, in the AD soil NO_3^--N concentrations were lower (P<0.05) at a soil ψ of −3 kPa, in both the acetate (88 μg NO_3^--N g^{-1} soil) and glucose (79 μg NO_3^--N g^{-1} soil) treatments, when compared to the control treatment (242 μg NO_3^--N g^{-1} soil), but this was not the case at −5 and −7 kPa (Table 3.3). Regardless of soil ψ and substrate treatment the NO_3^--N concentrations, on day 14, in the LU and LD soils ($\leq$ 60 μg NO_3^--N g^{-1} soil) were consistently an order of magnitude lower (P<0.001) than in the control ($\geq$130 μg NO_3^--N g^{-1} soil) treatment (Table 3.3).

Soil NH_4^+-N concentrations varied with soil type, being higher in the LU and LD soils than in the AD soil on days 3 and 14 (P<0.001, Table 3.2, 3.3) but concentrations did not differ with C substrate on either day. At a soil ψ of −3 kPa, the NH_4^+-N concentrations were higher than those at a soil ψ of −7 kPa with the exception of the AD soil on day 14 where no such effect occurred (P<0.05, Table 3.3).

Table 3.2 Values of soil surface pH, gravimetric water content (θ_g; %), dissolved organic carbon (DOC; µg g^{-1}), nitrate nitrogen (NO_3^--N; µg g^{-1}), and ammonium nitrogen (NH_4^+-N; µg g^{-1}) on day 3 for three levels of matric potential (-3, −5 and −7 kPa), three different substrates (acetate, glucose, water), and three soils (Ashley Dene dairy farm (AD), Lincoln University dairy farm (LU), and Lincoln University Demonstration Farm (LD)). All values shown are mean ± SD, n=4.

Day 3		AD			LU			LD		
		−3 kPa	−5 kPa	−7 kPa	−3 kPa	−5 kPa	−7 kPa	−3 kPa	−5 kPa	−7 kPa
pH	Acetate	6.4±0.2	6.3±0.1	6.4±0.3	6.8±0.4	6.7±0.4	6.8±0.3	7.2±0.4	6.9±0.3	7.1±0.6
	Glucose	6.2±0.1	5.9±0.1	5.9±0.2	6.3±0.5	6.3±0.3	6.3±0.1	6.5±0.6	6.3±0.5	6.3±0.6
	Water	5.6±0.1	5.6±0.1	5.7±0.2	5.5±0.1	5.4±0.0	5.6±0.3	5.6±0.0	5.4±0.2	5.5±0.3
θ_g	Acetate	38±1	33±1	30±1	47±1	45±1	44±1	48±5	48±1	47±2
	Glucose	38±1	34±1	29±1	47±6	45±1	45±1	51±1	48±1	47±2
	Water	38±1	32±1	29±0	48±2	46±2	45±2	50±1	48±1	46±6
DOC	Acetate	95±33	66±35	77±46	224±31	254±34	145±52	236±60	289±51	183±57
	Glucose	78±19	216±50	50±22	254±55	197±22	143±36	244±45	150±38	138±15
	Water	70±18	40±14	41±17	85±16	105±28	90±23	156±32	139±31	147±20
NO_3^--N	Acetate	290±17	329±22	317±22	255±22	317±66	324±51	349±23	277±69	336±45
	Glucose	317±28	310±36	278±28	372±85	303±39	279±22	218±59	362±30	234±17
	Water	311±59	361±42	339±48	319±37	246±57	358±38	250±45	276±17	305±17
NH_4^+-N	Acetate	3.9±1.6	1.6±0.5	0.4±0.2	13.3±2.1	14.1±2.8	7.9±1.6	15.6±1.4	9.4±0.8	10.4±1.5
	Glucose	4.3±0.2	1.3±0.5	0.3±0.1	11.6±3.2	13.0±3.0	6.7±0.8	16.5±2.6	8.8±1.3	4.4±2.1
	Water	4.1±1.0	1.1±0.5	0.4±0.2	19.0±1.5	13.6±1.5	7.0±1.4	19.2±1.4	13±3.0	10.4±1.2

Table 3.3 Values of soil surface pH, gravimetric water content (θ_g; %), dissolved organic carbon (DOC; μg g^{-1}), nitrate nitrogen (NO_3^--N; μg g^{-1}), and ammonium nitrogen (NH_4^+-N; μg g^{-1}) on day 14 for three levels of matric potential (-3, −5 and −7 kPa), three different substrates (acetate, glucose, water), and three soils (Ashley Dene dairy farm (AD), Lincoln University dairy farm (LU), and Lincoln University Demonstration Farm (LD)). All values shown are mean ± SD, n=4.

Day 14		AD			LU			LD		
		−3 kPa	−5 kPa	−7 kPa	−3 kPa	−5 kPa	−7 kPa	−3 kPa	−5 kPa	−7 kPa
pH	Acetate	8.8±0.1	8.8±0.1	8.7±0.1	8.8±0.1	8.7±0.1	8.8±0.1	8.8±0.0	8.7±0.1	8.7±0.2
	Glucose	8.0±0.3	7.7±0.5	7.1±0.1	8.3±0.3	8.3±0.2	7.9±0.4	8.2±0.5	8.3±0.1	8.0±0.1
	Water	6.0±0.2	5.7±0.1	5.8±0.1	5.3±0.1	5.6±0.4	5.6±0.1	5.8±0.2	5.9±0.6	5.6±0.1
θ_g	Acetate	39±3	34±1	30±2	48±1	46±1	44±1	50±1	48±1	47±3
	Glucose	39±2	34±2	30±1	47±2	46±2	47±2	49±3	48±1	44±6
	Water	39±1	34±1	29±1	49±1	46±1	43±1	49±2	48±1	47±2
DOC	Acetate	265±47	180±33	299±4	690±70	677±50	526±36	550±21	789±72	601±92
	Glucose	108±21	68±6	69±7	391±88	289±19	296±102	520±17	416±21	395±65
	Water	43±6	24±6	39±7	91±19	90±24	92±12	188±39	161±10	176±37
NO_3^--N	Acetate	88±14	248±28	193±15	13±1	22±9	60±22	9±2	27±8	52±23
	Glucose	79±14	143±11	174±33	5±1	9±5	16±8	3±1	9±4	11±3
	Water	242±37	248±25	230±27	206±26	195±35	229±15	130±13	199±3	165±16
NH_4^+-N	Acetate	3.0±1.3	1.5±0.1	1.4±0.5	11.3±2.2	8.4±1.2	2.0±0.8	13.7±0.8	4.5±1.6	2.4±1.2
	Glucose	0.7±0.1	0.4±0.2	0.8±0.4	5.4±1.1	3.0±0.7	2.0±0.5	6.8±0.6	7.4±0.4	5.4±0.8
	Water	1.0±0.2	0.6±0.1	1.2±0.6	15.7±2.4	5.4±0.6	4.1±0.3	9.7±0.6	5.1±0.5	7.2±0.6

3.4.2 N_2O and N_2 emissions

For all treatments, N_2O emissions generally peaked between days 3 and 5 (Fig. 3.1). An exception was the less sensitive response to substrate addition, in terms of N_2O emissions, for the AD soil at −7 kPa (Fig. 3.1; Table S3.2).

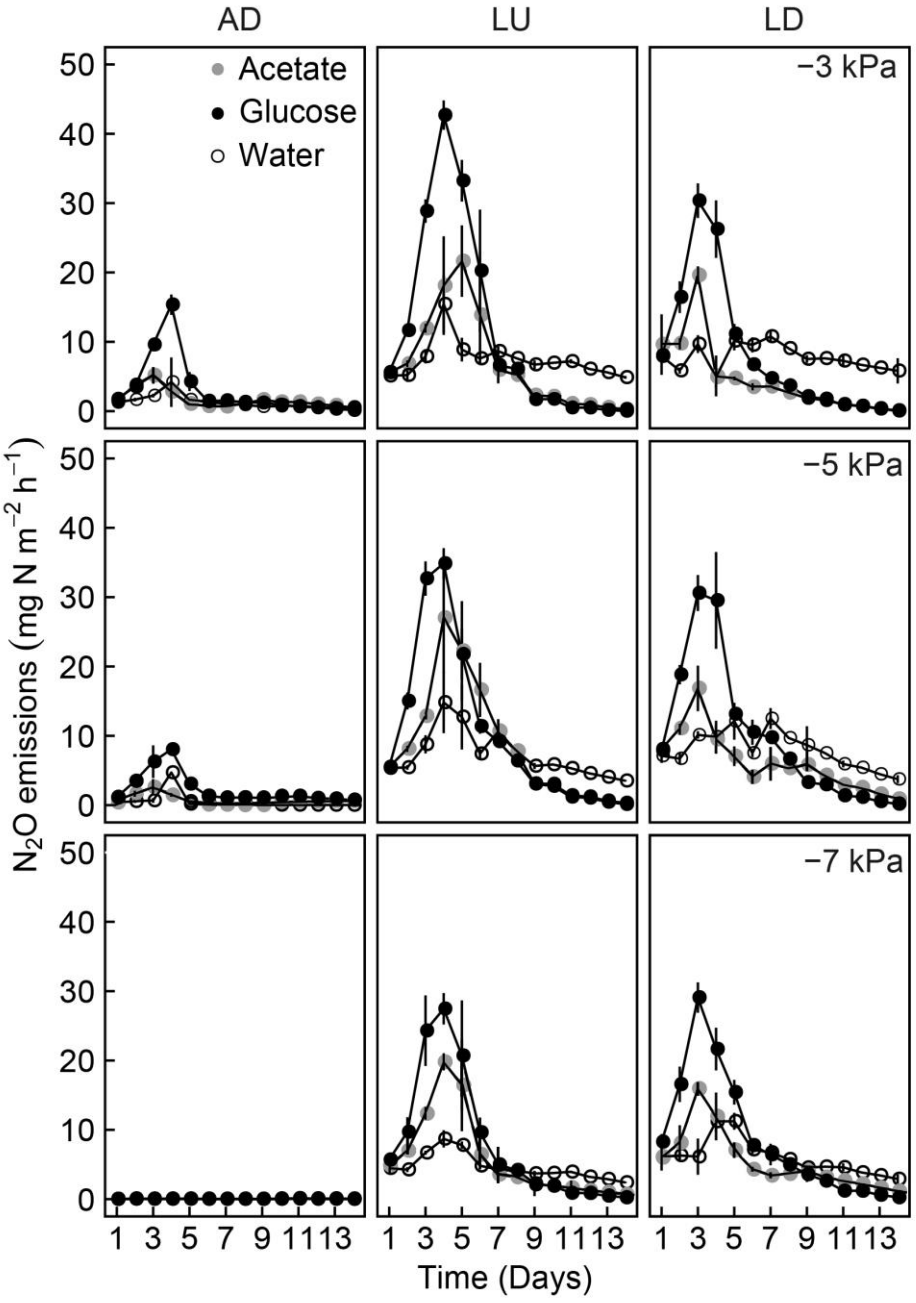

Fig. 3.1 Soil nitrous oxide emissions over the 14 day measurement period. Soils were treated with three levels of soil matric potential (−3, −5, and −7 kPa), and three different substrates (acetate, glucose, and water). Soils were sampled from three sites: Ashley Dene dairy farm (AD), Lincoln

University dairy farm (LU), and Lincoln University Demonstration Farm (LD). Values are means of four replicates (± SD), n=4.

The N_2O peak emissions were generally highest for soils treated with glucose (P<0.05; Table S3.2). Over the first seven days, N_2O emissions were higher for the LU soil at −3 kPa compared with values for the LD and AD soils when glucose substrate was applied (Fig. 3.1). Mean N_2O emissions across soil ψ for the LU and LD soils were higher than those for the AD soil (P<0.01) over the first seven days (Fig. 3.1). From day 8, N_2O emissions from the LU and LD control treatments were higher than those from the acetate and glucose treatments for these soils, and higher than from any AD soil treatment over this time (P<0.05; Fig. 3.1).

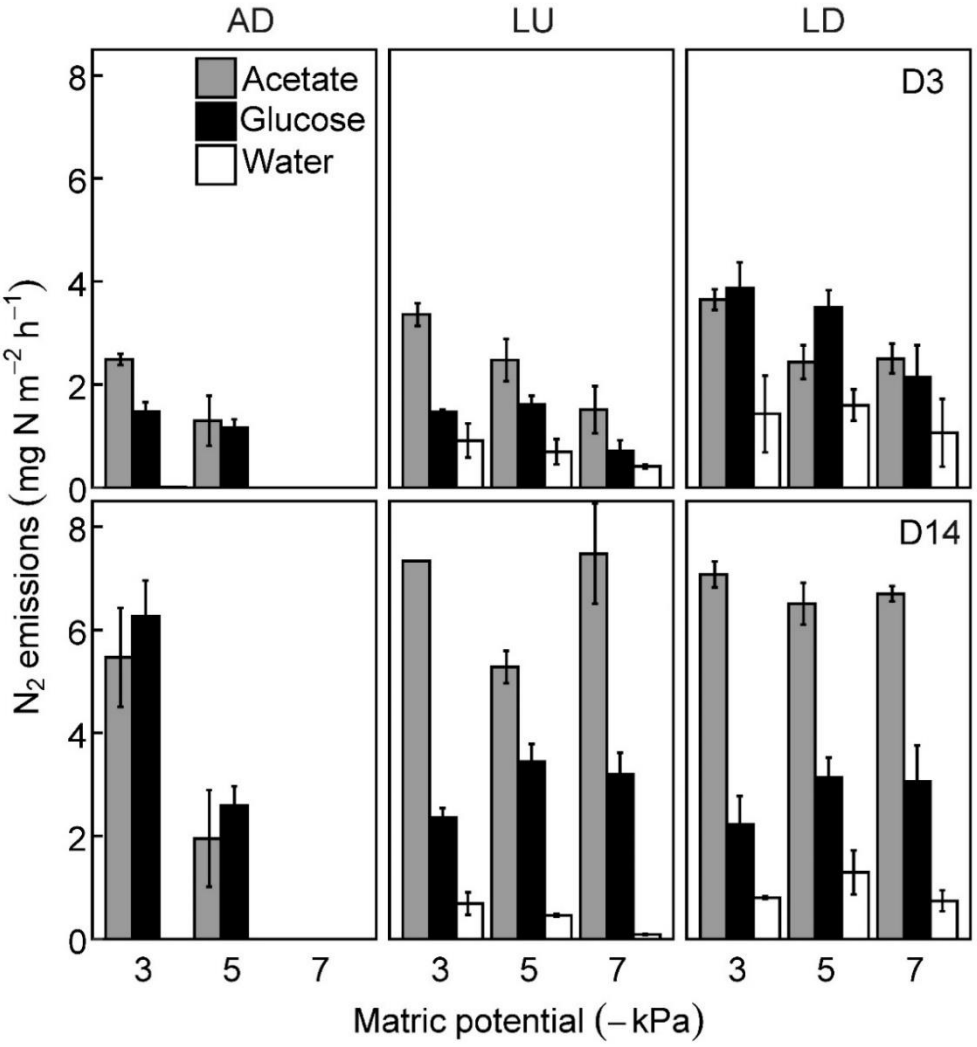

Fig. 3.2 The effects of substrate addition and soil matric potential on N_2 emissions on day 3 and 14 for the three soils at Ashley Dene dairy farm (AD), Lincoln University dairy farm (LU), and Lincoln University Demonstration Farm (LD). Values are means of four replicates (± SD), n=4.

After 14 days, mean cumulative N_2O-N emissions across substrate and soil ψ treatments from the LU and LD soils did not differ from each other but these were higher than those from the AD soil (P< 0.001; Table S3.3). Glucose addition resulted in higher cumulative N_2O emissions (P<0.05) when averaged across soil type and soil ψ treatments. However, soil ψ treatment did not affect cumulative N_2O emissions when averaged across soil type and substrate treatments.

On day 3, regardless of soil type and soil ψ treatments, N_2 emissions were higher with glucose and acetate substrate addition than those with water addition (P< 0.05, Fig. 3.2); higher from the LD soil than that for LU or AD soils when averaged across treatments (P< 0.05); and higher from the −3 kPa treatment (P< 0.05) than those values at −7 kPa with neither of these treatments differing from the values for the −5 kPa treatment.

On day 14, there were no effects of soil ψ on mean N_2 emissions when averaged across soil type and substrate treatments. But substrate type affected N_2 emissions with higher emissions of N_2 under acetate than glucose on day 14, with an interaction between substrate and soil resulting in higher N_2 emissions from the acetate-treated LU and LD soils than from the glucose-treated LU and LD soils (P<0.01), but this did not occur in the AD soil (Fig. 3.2). In turn, the N_2 emissions from glucose-treated LU and LD soils were higher than those from water-treated soils P<0.05; Fig. 3.2). Averaged across soil ψ potential the acetate:glucose N_2 emission ratio was 2.56 ± 0.75 (Mean ± SD), 2.35 ± 0.81, and 0.83 ± 0.31 for the LD, LU, and AD soils, respectively.

Carbon substrate type affected the N_2O:(N_2O+N_2) emission ratio at day 3 (Fig. 3.3), with higher (P<0.05) values under glucose and water, 0.91 and 0.90 respectively, than those for soils treated with acetate (0.81). Soil type affected the N_2O:(N_2O+N_2) emissions ratio on day 3 with a higher N_2O:(N_2O+N_2) ratio for the LU (0.91) and LD (0.87) soils, than the ratio for the AD soil (0.80; P<0.05). On day 14, the N_2O:(N_2O+N_2) emission ratios for soils treated with acetate (0.10) or glucose (0.07) were lower than those for the water-treated soil (0.86, P<0.05). The N_2O:(N_2O+N_2) emission ratio was highest under water-treated LU and LD soils and lowest under glucose-treated LU and LD soils on day 14 (P<0.05; Fig. 3.3). The AD soil N_2O:(N_2O+N_2) emission ratio did not vary as a result of glucose or acetate treatment at this time. On day 14, averaged across substrate and soil ψ, soil type had no significant effect on the N_2O:(N_2O+N_2) emission ratio. Similarly, soil ψ had no effect on the N_2O:(N_2O+N_2) emission ratio on either day 3 or 14.

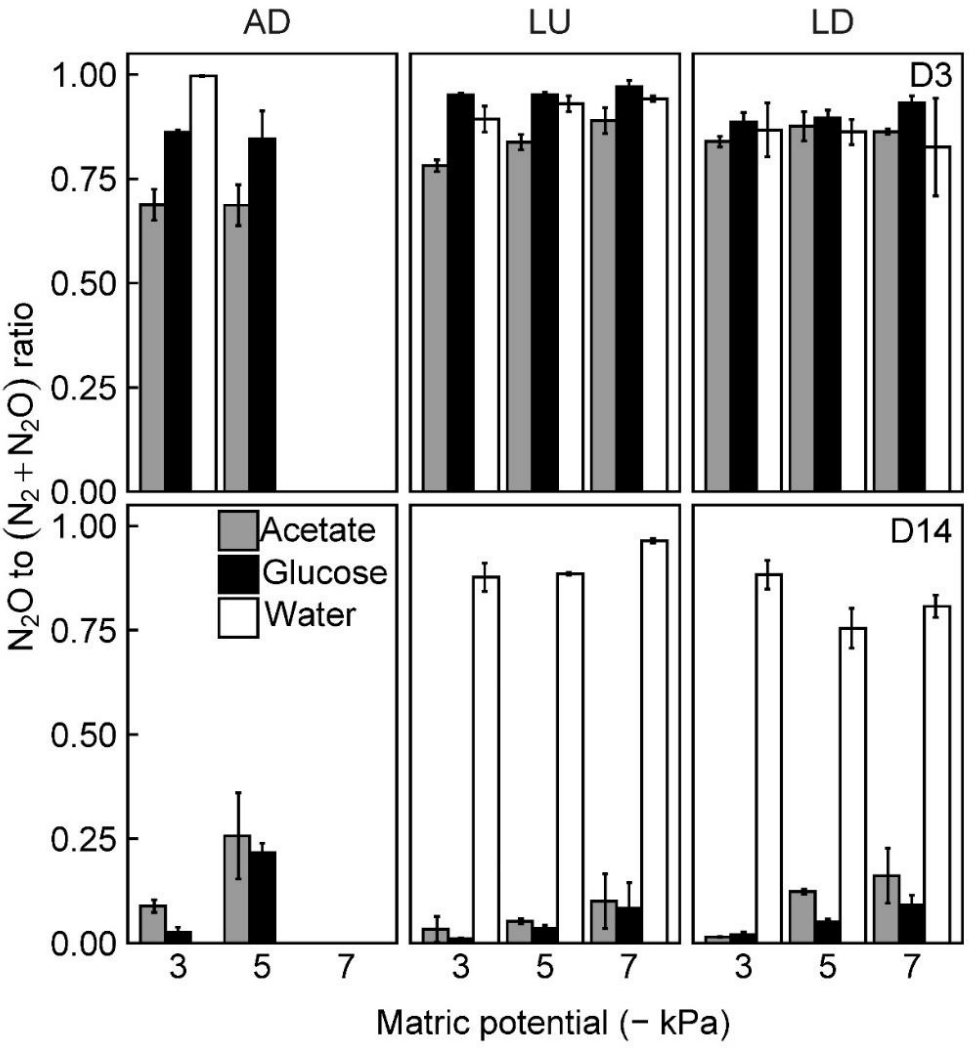

Fig. 3.3 The effects of substrate additions and soil matric potential on the ratio of N_2O:(N_2 + N_2O) on day 3 and 14. Soils were sampled from three sites: Ashley Dene dairy farm (AD), Lincoln University dairy farm (LU), and Lincoln University Demonstration Farm (LD). Values are means of four replicates (± SD), n=4.

3.4.3 Soil CO_2 emissions

Based on the model (Equation 3.1), the response of CO_2 emissions to glucose or acetate addition was best fitted by an exponential curve (Fig. 3.4). An exception to this was the AD soil treated with acetate at a soil ψ of −3 kPa where steady state was not reached.

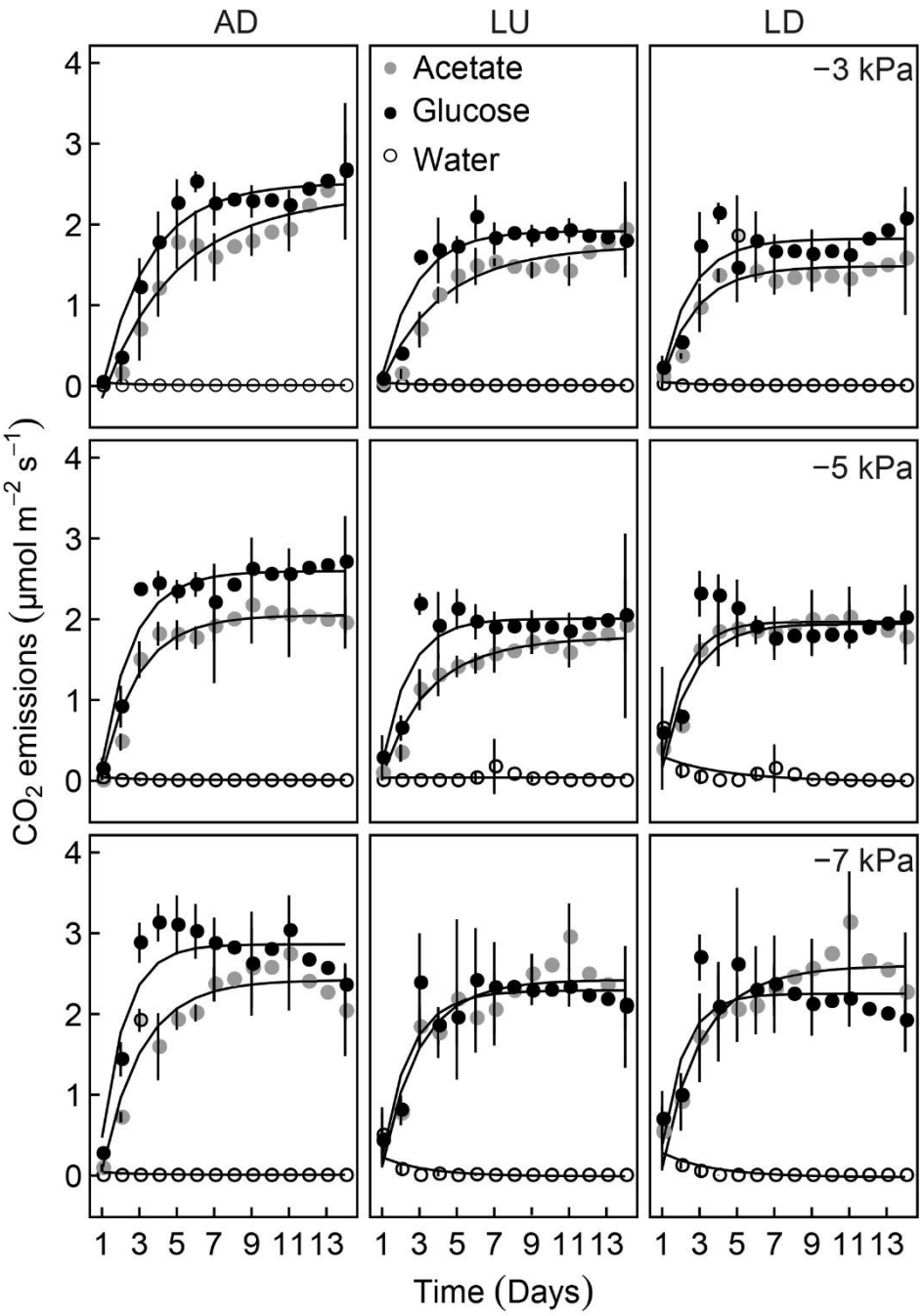

Fig. 3.4 Soil respiration rates over the 14 days. Soils were treated with three levels of soil matric potential (−3, −5, and −7 kPa), and three different substrates (acetate, glucose, and water). Soils were sampled from three sites: Ashley Dene dairy farm (AD), Lincoln University dairy farm (LU), and Lincoln University Demonstration Farm (LD). Values are means of four replicates (± SD), n=4. Solid lines represent the exponential curve yielded by non−linear mixed−effect models according to Equation 3.1.

Steady state CO_2 emissions in the other treatments at −3 kPa did not differ with soil type or substrate treatment (Table 3.4). With the exception of the AD soil treated with glucose (2.6 ± 0.2

μmol m^{-2} s^{-1}, P<0.05), where maximum steady state CO_2 emissions occurred at −5 kPa, and the LU soil treated with acetate (1.6 ± 0.0 μmol m^{-2} s^{-1}, P<0.05) where the minimum value of steady state CO_2 emissions occurred at −5 kPa there were no differences in the magnitude of steady state CO_2 emissions at −5 kPa due to soil or substrate (Table 3.4). Steady-state CO_2 emissions were highest in the AD soil treated with glucose at −7 kPa (Table 3.4, P<0.05), otherwise there were no other treatment effects on the magnitude of steady state CO_2 emissions at −7 kPa.

The rate at which steady state CO_2 emissions were reached at −3 kPa generally did not differ with soil type or substrate treatments, the exception being the acetate-treated LU soil which took longer to reach steady state CO_2 emissions than in the LD glucose-treated soil (P<0.05; Table 3.4). At −5 kPa the acetate-treated LU and AD soils required more time to reach a steady state of CO_2 emissions than the LD glucose-treated soil (P<0.05; Table 3.4). An increase in the time period to reach a steady state of CO_2 emissions was also observed at −7 kPa for the acetate-treated AD soil when compared with both the glucose treated AD soil (P<0.05; Table 3.4). There was generally no effect of soil ψ on the time required to reach a steady state of CO_2 emissions, the only exception being a higher value steady state value at −7 kPa than at −3 kPa in the acetate treated LU soil (P<0.05; Table 3.4).

Generally, over 14 days, cumulative CO_2-C emissions from soils amended with glucose were higher than those with no amendment, ranging from 27.9 ± 1.6 to 36.6 ± 1.8, 23.0 ± 0.4 to 28.7 ± 1.6, and 22.5 ± 1.3 to 29.2 ± 3.6 g C m^{-2} for the AD, LU, and LD soils, respectively (P<0.05; Table 3.4). Cumulative CO_2-C emissions from the water treatment (control) were lower than those in the acetate and glucose treatments for all soils and soil ψ treatments (P<0.05; Table 3.4).

Averaged across other treatments there was no treatment effect of soil type or soil ψ treatments (Table S3.3). A soil type by substrate interaction resulted in higher cumulative CO_2 emissions from the glucose-treated AD soil when compared to all other acetate- and glucose-treated soils. An exudate by soil ψ treatment interaction resulted in higher (P<0.05) cumulative CO_2 emissions from the acetate- and glucose-treated soil at −7 kPa when compared to all other acetate- and glucose treated soils (Table S3.3).

Table 3.4 Parameters values for modelled soil respiration rate (*a*, is the steady−state value of the soil respiration rate, µmol m^{-2} s^{-1}; *c* characterises the exponential decay rate, (m^2 s µmol^{-1}) day^{-1}) from Equation 3.1. Treatments are three levels of matric potential (-3, −5 and −7 kPa), and three types of substrate (acetate, glucose, and water), three soils (Ashley Dene dairy farm (AD), Lincoln University dairy farm (LU), and Lincoln University Demonstration Farm (LD)). All values shown are mean ± SD, n=4. Same parameter value was compared with a Tukey *HSD* test. Parameters values of the water treatment were significantly different from all other treatments and thus were excluded. Letters indicate significant differences (*P* < 0.05) between treatments for the 3-way interaction between carbon substrate, soil, and soil matric potential.

		a			c		
		−3 kPa	−5 kPa	−7 kPa	−3 kPa	−5 kPa	−7 kPa
AD	Acetate	1.38±1.26	2.06±0.23 cdef	2.36±0.16 bc	0.29±0.24	0.51±0.06 bc	0.52±0.06 bc
	Glucose	1.60±1.71 abcdefg	2.60±0.17 ab	3.03±0.29 a	0.34±0.27 abcd	0.61±0.08 ab	0.70±0.05 a
LU	Acetate	1.68±0.07 efg	1.62±0.03 fg	2.16±0.13 bcd	0.39±0.05 c	0.48±0.05 bc	0.58±0.07 ab
	Glucose	1.96±0.06 cdefg	2.08±0.20 cde	2.29±0.32 bcd	0.52±0.03 bc	0.62±0.02 ab	0.60±0.06 ab
LD	Acetate	1.55±0.05 g	1.98±0.16 cdefg	2.33±0.11 bcd	0.50±0.03 bc	0.59±0.03 ab	0.60±0.12 ab
	Glucose	1.87±0.14 defg	2.06±0.12 cdef	2.29±0.28 bcd	0.58±0.06 ab	0.70±0.02 a	0.68±0.06 a

3.4.4 Factors affecting CO_2 and N_2O emissions

Pooling data by C substrate across soil type and soil ψ treatments showed cumulative N_2O-N emissions declined exponentially with increasing D_p/D_o, with 67% and 65% of the variation in cumulative N_2O-N losses explained for soils treated with glucose and acetate, respectively (Fig. 3.5). In contrast, pooling the data in a similar manner showed a positive linear relationship, between D_p/D_o and cumulative CO_2 emissions, with 47% and 21% of the variation explained for glucose and acetate, respectively (Fig. 3.5).

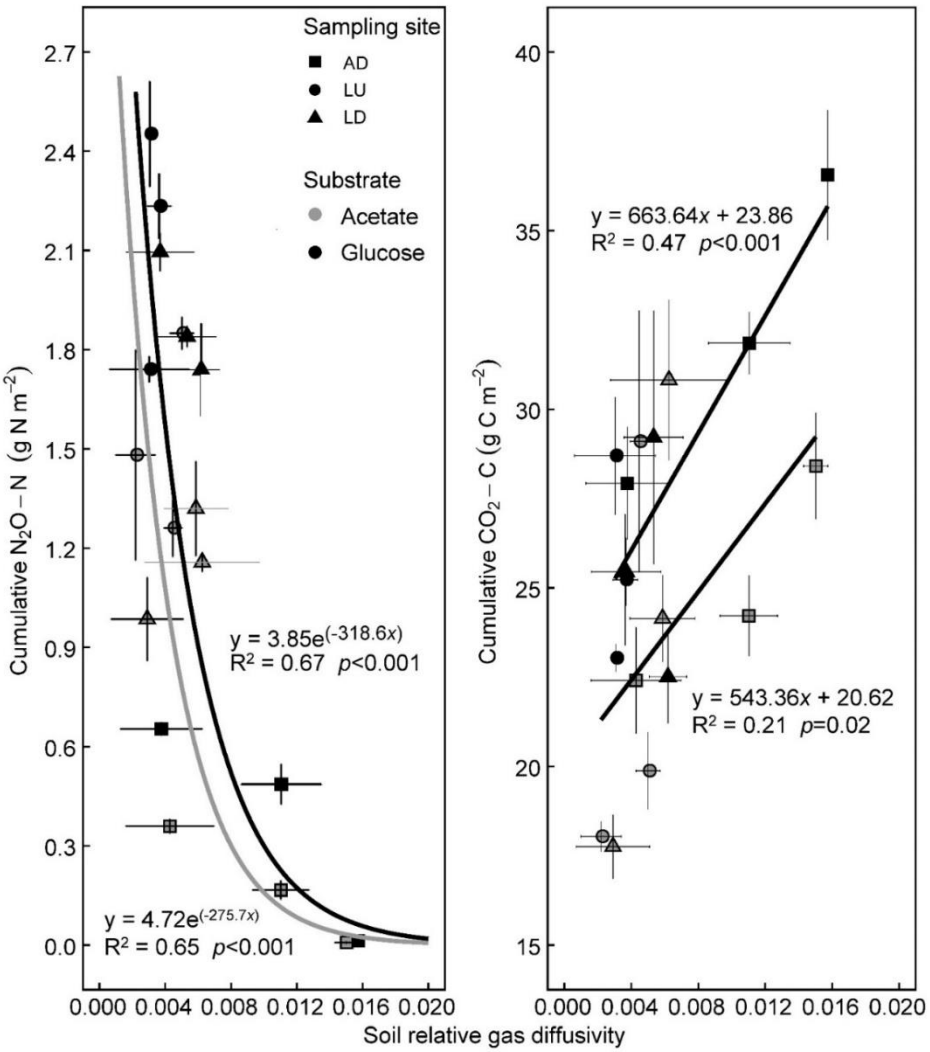

Fig. 3.5 The relationship between cumulative N_2O–N emissions, cumulative CO_2–C emissions and soil relative gas diffusivity. Values are means of four replicates (± SD), n=4. Soils were treated with two different substrates (acetate, and glucose). Soils come from the Ashley Dene dairy farm (AD), Lincoln University dairy farm (LU), and Lincoln University Demonstration Farm (LD).

3.5 Discussion

Soil WFPS and diffusivity data show that soil water content conditions were suitable for denitrification, with diffusivity values < 0.006 and WFPS > ca. 80% (Linn and Doran, 1984; Balaine et al., 2013), with the exception of the AD soil which, due to its higher sand content, held less water at matric potentials of −5 kPa and −7 kPa. The positive responses, by way of N_2O and N_2 production, following application of NO_3^- and C substrates indicate denitrification was the dominant pathway responsible for N_2O and N_2 production. Under anaerobic conditions, heterotrophic denitrifiers produce N_2O and ultimately N_2, following the stepwise reduction of the obligate denitrification intermediaries: NO_3^-, nitrite (NO_2^-), and nitric oxide (NO). Dissimilatory nitrate reduction to ammonia (DNRA) can also produce N_2O under anaerobic conditions in grassland soils (Friedl et al., 2018). However, DNRA is unlikely to have contributed significantly to N_2O emissions since concentrations of NH_4^+ remained relatively low. Nitrifiers may also produce N_2O following the anaerobic oxidation of hydroxylamine, under hypoxia via nitrifier-denitrification, or through biotic and abiotic transformations of nitrification intermediaries (Stein, 2019). Both the low level of NH_4^+ substrate available, a precursor to hydroxylamine, and the low O_2 levels (hypoxic conditions) imply anaerobic oxidation of hydroxylamine did not make a significant contribution to the N_2O emissions.

Peak N_2O emissions at ~3 days after substrate addition are consistent with the result of Samad et al. (2016) who examined 13 grassland soils from Ireland and New Zealand that were wetted up and amended with NO_3^- before undergoing anaerobic incubation. Upon commencement of the anaerobic incubation, production of NO, N_2O and N_2 all occurred with N_2O production generally peaking at ca. 90 hours and N_2 peaking after this time. In the current experiment, higher N_2O emissions occurred under glucose and acetate, compared to the emissions with water applied, as a consequence of the higher supply of electrons. The lower rate of N_2O production (-5 kPa), or lack of N_2O and N_2 production (-7 kPa) in the AD soil, can be attributed to conditions becoming too aerobic for denitrification, as shown by the higher diffusivity values, which in turn explains the lower NH_4^+ concentrations observed in this treatment, most likely the result of nitrification. The effect of soil texture on soil aeration is further supported by the relationship between cumulative N_2O and diffusivity, where increases in cumulative N_2O emissions aligned with a lower values of relative diffusivity (ca. <0.006) previously shown to induce N_2O emissions (Balaine et al., 2013). This was reflected in the absence of (-7 kPa), or relatively lower (-5 kPa), N_2 emissions from the AD soil, again likely the result of the higher diffusivity in the AD soil (Balaine et al., 2016) Thus, in support of the first hypothesis increasing soil ψ caused D_p/D_o to decline, invoking greater rates of denitrification.

Petersen et al. (2008) noted that increased consumption of O_2 as a result of an enhanced bioavailable C supply could increase the anoxic zone within a soil. Interestingly, the utilisation of the applied C substrates in the AD soil as evident from the CO_2 emissions, which were comparable in magnitude to those from the LU and LD soils, was not sufficient to induce anaerobic conditions at −5 and −7 kPa in the AD soil based on relative N_2O emissions. Thus, the diffusivity of the AD soil at −5 and −7 kPa helped maintain the relatively low N_2O emissions.

Despite anaerobic conditions persisting at −3 kPa in the AD soil, as shown by the relative diffusivity value, the N_2O emissions with substrate addition remained relatively low when compared to those for the LU and LD soils. This suggests factors other than anaerobicity were responsible for the lower N_2O emissions at −3 kPa in the AD soil. Differences in the microbial community composition or the way in which the specific soil's microbial community utilised the applied C substrate may explain the lower N_2O emissions in the AD soil compared to values for the LU and LD soils. For example, Giles et al. (2017) found that 120 hours after a single input of glucose, glutamine or citric acid, differences in the N_2O and N_2 emissions resulted from differences in substrate use efficiency. In a study of 13 grassland soils, Samad et al. (2016) found that the rate of soil denitrification was also closely linked to anoxic C-mineralisation (r^2 = 0.89), measured for 40 hours after removal of oxic conditions. Thus, despite substrate addition, it is also possible that the lower N_2O emissions observed in the AD soil at −3 kPa could result from the lower organic matter content of the AD soil and potentially differences in the quality or quantity of the DOC.

Nitrous oxide emissions under glucose and acetate addition declined by day 14 due to increasing N_2O reductase activity. Soil NO_3^- concentrations decreased over time and given that NO_3^- is the preferred electron acceptor to N_2O (Giles et al., 2012), this will have favoured N_2O reduction. For example, after applying organic substrates Senbayram et al. (2012) found that the transformation of N_2O to N_2 was more rapid once soil NO_3-N concentrations fell below 20 mg kg^{-1} soil. At day 14, this was the case for the LU and LD soils treated with glucose at all matric potentials, and for the LU and LD soils treated with acetate at −3 kPa. Similarly, the increase in soil pH over time will have favoured N_2O reductase activity (Firestone and Davidson, 1989; Samad et al., 2016).

It was hypothesised that, as previously found by (Morley et al., 2014), acetate would enhance N_2O reduction to N_2 relative to glucose regardless of soil type. When averaged across all treatments on day 3 our results confirm this hypothesis (Fig. 3.3), with a lower N_2O:(N_2O+N_2) ratio observed under acetate (0.81) than glucose (0.91). This effect was not present at day 14 due to the diminished production of N_2O and the dominance of N_2 as a denitrification product as noted above. Previously, Paul et al. (1989) and Morley et al. (2014) showed that the efficiency of N_2O reduction to N_2 was

substrate-dependent. It has been suggested that acetate is more efficient than glucose in promoting N_2O reduction, possibly due to the differential metabolism of glucose and acetate, with acetate entering directly the tricarboxylic acid (TCA) cycle (Gunina et al., 2014), which produces compounds directly linked to the electron transport chain (Gottschalk, 1986). While the dominance of N_2 production at day 14 precluded observing the possible effect of acetate on the N_2O:(N_2O+N_2) ratio at this time the more than 2-fold higher emissions of N_2 under the acetate-treated LU and LD soils, compared with the glucose-treated LU and LD soils, show that not only did acetate enhance N_2O reduction but it also enhanced the overall rate of denitrification on 14 days.

Low molecular weight organic compounds may be used by microbes and decomposed to CO_2 sorbed onto mineral surfaces or leached. Glucose has been reported to have a similar sorptive affinity and carbon-use efficiency as acetate (Keiluweit et al., 2015; Sokol et al., 2019). Both are rapidly utilised within minutes (Hill et al., 2008; Fischer and Kuzyakov, 2010). The relative magnitude of sorption varies with soil type (Jagadamma et al., 2012). Thus, the fates of glucose and acetate applied to soil may differ. For example, Gunina et al. (2014) showed that, under non-saturated soil conditions, similar initial uptakes of glucose and acetate by soil microorganisms occurred after 10 days, but more glucose ^{13}C than acetate ^{13}C was recovered from the extractable microbial biomass. Sugars are metabolised by microbes via glycolysis prior to glucose-C being incorporated into cell components or entering the TCA cycle (Bore et al., 2019) and glucose is recognised as providing the main source of C for a wide range of microbial communities (Paterson et al., 2007) providing more energy than acetate for microbial processes (Paul et al., 1989). However, glucose efficiency as a denitrification C substrate may decline if fermentative bacteria compete with denitrifiers for C (Paul et al., 1989). Given that acetate is non-fermentable (van den Berg et al., 2017) the lower N_2 emissions observed on day 14 in the LU and LD soils under glucose may have been the result of greater microbial competition for glucose. The fact the glucose-treated AD soil had similar N_2 emissions to the acetate-treated soil at day 14 again shows that the microbial community in the AD soil was also responding differently to substrate addition with respect to the LU and LD soils and potentially had a lower fraction of the community that was capable of fermentative competition for glucose. Potentially, the lower soil water holding capacity of the AD soil may have selected for a microbial community containing less fermentative microbes.

It was hypothesised that the CO_2 emissions from C substrates would not differ due to soil type. This was not the case. The fact the AD soil did not reach steady state CO_2 emissions at −3 kPa, despite comparable diffusivity to the LU and LD soils at this soil ψ, indicates the microbial pool utilising acetate was still growing, and this is also reflected in the lower denitrification emissions at −3 kPa in

the AD soil at day 3. The lower soil C concentration in the AD soil, reflected in the lower DOC concentrations in the water treatment, may have also resulted in a lower microbial pool being initially present. The enhanced utilisation of glucose in the AD soil at −5 and −7 kPa aligns with the enhanced diffusivity of these treatments with an increased O_2 supply driving the CO_2 emission response in the AD soil in these treatments.

In addition to substrate, a further factor compounding soil CO_2 emissions responses is the antecedent soil C quantity and quality. Besides substrate decomposition, CO_2 emissions may also result from substrate-induced priming stimulating the decomposition of antecedent soil C (Schimel and Weintraub, 2003; Shahbaz et al., 2018). Thus, it is also possible that the observed CO_2 emissions responses were partially due to priming effects. However, this study did not aim to determine substrate effects on the priming contributions to CO_2 emissions. Future studies are required to examine this with respect to N_2O and N_2 emissions in order to better clarify potential interactions between soil type and C substrate with respect to the N_2O:(N_2O+N_2) emission ratio and gross denitrification rates.

The positive response of soil CO_2 emissions to decreasing soil ψ observed here (Fig. 3.5) is in agreement with Groffman and Tiedje (1991) who determined the response of soil CO_2 emissions across the full range of soil water content to be parabolic. For both substrates this positive response was driven strongly by the highest cumulative CO_2 emissions. Samad et al. (2016) found CO_2 emissions under aerobic conditions in grassland soils matched with high rates of C mineralisation. High CO_2 emissions occurred in the AD soil at the highest diffusivity levels in the AD soil (−5 and −7 kPa) where N_2O and N_2 fluxes were relatively low or non existent. Hence, overriding the availability, and ability, of soil C substrate addition, to denitrify NO_3^- is the requirement for suitable anaerobic conditions as dictated by soil diffusivity.

3.6 Conclusions

Emissions of CO_2 and N_2O over 14 days, along with N_2 emissions, were successfully measured from three NO_3^- amended soils held at varying soil matric potential that received daily acetate and glucose additions. Soil matric potential and soil texture determined soil relative gas diffusivity, which in turn influenced denitrification and CO_2 emissions. Carbon substrate regulated denitrification products: acetate initially produced lower peak N_2O emissions and lower N_2O:(N_2O+N_2) ratios than glucose. By day 14 the denitrification emissions were dominated by N_2, with soils with higher organic matter content and finer texture having 2-fold greater N_2 emissions under acetate compared with glucose: it is speculated that by day 14 the competition for glucose, between fermentative microbes and

denitrifiers, resulted in lower N_2 emissions via denitrification when compared to acetate. Soil type and substrate influenced the time taken to reach steady state for CO_2 emissions and the maximum rate of CO_2 emissions, due to differences in soil gas diffusivity and potentially differences in the soil microbial communities present.

Supplemental Table S3.1 Values of soil relative gas diffusivity. Three soils at Ashley Dene dairy farm (AD), Lincoln University dairy farm (LU), and Lincoln University Demonstration Farm (LD). All values shown are mean ± SD, n=9.

	−3 kPa	−5 kPa	−7 kPa
AD	0.0040±0.0023	0.0110±0.0019	0.0154±0.0028
LU	0.0026±0.0023	0.0043±0.0010	0.0037±0.0018
LD	0.0045±0.0024	0.0048±0.0022	0.0058±0.0025

Supplemental Table S3.2 Maximum N_2O emissions for three levels of matric potential (-3, −5 and −7 kPa), and three different substrates (acetate, glucose, water), maximum N_2O emissions from three soils at Ashley Dene dairy farm (AD), Lincoln University dairy farm (LU), and Lincoln University Demonstration Farm (LD). The day number represents the time peak emissions occurred. All values shown are mean ± SD, n=4. Parameter values were compared with a Tukey *HSD* test.

		Day			Maximum N_2O emission (mg N m^{-2} h^{-1})		
		−3 kPa	−5 kPa	−7 kPa	−3 kPa	−5 kPa	−7 kPa
AD	Acetate	3	3	2	5.2±1.2 mnop	2.6±1.2 op	0.09±0.00 p
	Glucose	4	4	11	15.4±1.5 hijkl	8.0±0.9 lmnop	0.1±0.00 p
	Water	3	3	11	4.9±2.8 mnop	5.1±4.7 mnop	0.05±0.00 p
LU	Acetate	5	5	4	21.6±5.2 efgh	31.2±3.4 bcd	20.5±2.2 fghi
	Glucose	4	4	3	42.7±2.1 a	35.2±1.9 ab	29.1±1.4 bcdef
	Water	4	3	4	36.0±4.8 ab	17.7±1.5 ghij	8.8±1.2 klmno
LD	Acetate	3	3	3	19.6±1.3 ghij	16.8±3.3 hijk	15.9±0.9 hijkl
	Glucose	3	4	3	30.4±2.5 bcd	33.9±2.4 bc	29.1±2.2 bcdef
	Water	7	5	4	11.1±0.6 jklmno	12.8±1.8 ijklm	11.5±1.4 jklmn

Supplemental Table S3.3 Cumulative N_2O and CO_2 emissions over 14 days for three levels of matric potential (-3, −5 and −7 kPa), and three different substrates (acetate, glucose, water), for soils from Ashley Dene dairy farm (AD), Lincoln University dairy farm (LU), and Lincoln University Demonstration Farm (LD). All values shown are mean ± SD, n=4. Parameter values were compared with a Tukey *HSD* test.

		Cumulative N_2O-N loss (g N m^{-2})			Cumulative CO_2-C loss (g C m^{-2})		
		−3 kPa	−5 kPa	−7 kPa	−3 kPa	−5 kPa	−7 kPa
AD	Acetate	0.36±0.02 no	0.17±0.03 op	0.01±0.00 p	22.4±1.5 ghij	24.2±1.1 efghi	28.4±1.5 cdef
	Glucose	0.65±0.02 m	0.49±0.06 mn	0.01±0.00 p	27.9±1.6 cdef	31.9±2.6 bc	36.6±1.8 a
	Water	0.27±0.04 nop	0.11±0.07 op	0.00±0.00 p	0.1±0.0 l	0.1±0.0 l	0.1±0.0 l
LU	Acetate	1.48±0.32 fghi	1.85±0.05 cd	1.26±0.09 hijk	18.0±0.4 jk	19.9±1.1 ijk	29.1±3.7 bcd
	Glucose	2.45±0.16 a	2.23±0.10 ab	1.74±0.04 def	23.0±0.4 ghi	25.2±1.8 defgh	28.7±1.6 bcde
	Water	1.91±0.11 cd	1.57±0.02 efg	1.00±0.03 kl	0.1±0.0 l	0.4±0.1 l	0.6±0.4 l
LD	Acetate	0.99±0.03 l	1.32±0.14 ghij	1.16±0.03 jkl	17.8.±0.9 k	24.1±1.2 fghi	30.8±2.2 bc
	Glucose	1.74±0.14 def	2.09±0.06 bc	1.84±0.03 cde	22.5±1.3 ghij	25.5±1.0 defg	29.2±3.6 bcd
	Water	1.67±0.04 def	1.71±0.17 def	1.30±0.02 ghij	0.1±0.0 l	1.2±0.9 l	0.8±0.5 l

Chapter 4 Nitrous oxide emissions from denitrification depend on the energy available from soil organic matter decomposition and added carbon substrates

4.1 Abstract

Carbon (C) substrate is critical for regulating denitrification, a process that results in nitrous oxide (N_2O) emissions from soil. The chemical form of C substrates also modifies the rate of soil organic matter (SOM) decomposition, termed the priming effect. However, the relationships between the priming effect and N_2O production from soil, in relation to nitrogen (N) and C supply, are not well known. We applied ^{13}C–labelled substrates (acetate, butyrate, glucose; 80 μg C g^{-1}), with water as a control, and ^{15}N–labelled N (300 μg N g^{-1} soil, potassium nitrate) to three different soils, and after 3 days measured the effects on the priming of SOM and sources of N_2O emission. Carbon substrate addition increased both CO_2 and SOM derived N_2O emissions in the presence of exogenous N. Emissions of CO_2 and N_2O from soils with added glucose (mean ± SD, 0.73 ± 0.13 μmol m^{-2} s^{-1} and 21.4 ± 12.1 mg N m^{-2} h^{-1}) were higher than those from soils treated with acetate (0.64 ± 0.11 μmol m^{-2} s^{-1} and 10.9 ± 6.5 mg N m^{-2} h^{-1}) or butyrate (0.61 ± 0.11 μmol m^{-2} s^{-1} and 11.0 ± 6.6 mg N m^{-2} h^{-1}), respectively. Acetate addition induced a stronger priming effect (0.07 ± 0.09 μmol m^{-2} s^{-1}) than that for glucose (0.02 ± 0.10 μmol m^{-2} s^{-1}), while butyrate addition resulted in negative priming (-0.09 ± 0.05 μmol m^{-2} s^{-1}). SOM derived N_2O emissions were relatively low from soils with butyrate addition (1.4 ± 1.5 mg N m^{-2} h^{-1}) compared with acetate (2.9 ± 2.3 mg N m^{-2} h^{-1}) or glucose (9.2 ± 4.5 mg N m^{-2} h^{-1}). However, we did not detect a clear relationship between priming effect and SOM derived N_2O emissions. Our results highlight the need to consider the nature of the C substrate when interpreting processes regulating SOM decomposition and N_2O emission source.

Keywords: carbon source; ^{15}N; organic acids; ^{13}C

4.2 Introduction

Addition of carbon (C) to agricultural soils may occur as inputs from dead plant material, exudates from roots and/or organic amendments. Many studies have reported changes in the rate of soil organic matter (SOM) decomposition following the addition of C inputs (Qiao et al., 2016; Hicks et al., 2019), known as priming (Kuzyakov et al., 2000). While studies have determined the effects of priming on soil carbon dioxide (CO_2) emissions (Qiao et al., 2014; Jones et al., 2018) and soil C stocks

(Clemmensen et al., 2013; Jia et al., 2017), much less is known about the effects of C inputs on nitrogen (N) cycling and, in particular, nitrous oxide (N_2O) emissions (Morley et al., 2014; Fisk et al., 2015). Mitigation of N_2O emissions from soils is a high priority for agricultural systems because they contribute 50−60% of total greenhouse gas emissions, globally (Ciais et al., 2013). Hence, there is a need for a deeper understanding of the effects of C substrates on N_2O and CO_2 emissions derived from SOM.

The lack of knowledge about the effects of C substrates on N_2O and CO_2 emissions is attributable partly to the complex interactions between C and N cycling in soils. Priming was defined by Kuzyakov et al. (2000) as short−term changes in the turnover of SOM caused by comparatively moderate treatments of the soil. Previous studies have reported positive (Hamer and Marschner, 2005; Jia et al., 2017), negative (Kuzyakov and Cheng, 2004; Hamer and Marschner, 2005) and neutral (Kuzyakov and Cheng, 2004) priming effects. A range of mechanisms have been proposed to explain priming, but no consensus has yet been reached (Kuzyakov et al., 2000; Cheng et al., 2014). Nitrogen availability can influence both the direction and magnitude of priming (Mason-Jones et al., 2018; Hicks et al., 2019) while C substrate addition can stimulate the rate of N immobilisation (Kirchmann and Lundvall, 1993; Fisk et al., 2015). Hence, N stoichiometry is most often invoked to explain these priming observations. The N mining hypothesis suggests that priming occurs after microbial demand for N increases as a consequence of increasing microbial biomass resulting from rapid metabolism of added labile substrates (Chen et al., 2014). Thus, the priming effect would be anticipated to be greater for soils with low N availability. It follows that priming effects could be reduced, or negative priming may be induced, by adding mineral N to the soil in the form of fertilisers (Fanin et al., 2015; Hicks et al., 2019) that alleviate nutrient limitations (Hamer and Marschner, 2005; Tian et al., 2016). However, microbial demand for N is insufficient to explain all priming observations (Hicks et al., 2019).

The direction and intensity of priming has been shown to depend on the chemical nature of C substrates within soils. Organic acids, such as oxalic acid, have been observed to result in stronger positive priming than glucose due to the solubilisation of mineral−protected SOM (Keiluweit et al., 2015), and increased N availability through net mineralisation from enhanced microbial activity (Yuan et al., 2018). However, Mason-Jones et al. (2018) observed that while N addition suppressed the rate of native C mineralisation, it did not greatly change the priming effects of C substrates.

Several studies have shown that the production of N_2O from nitrification and/or denitrification depends on the quantity and quality of C substrates (Paul et al., 1989; Murray et al., 2004; Morley et al., 2014). For example, the chemical nature and quantity of C available to soil denitrifiers can

regulate the efficiency of the N_2O reductase enzyme that changes the stoichiometric ratio between the dinitrogen (N_2) and N_2O produced (Morley and Baggs, 2010; Morley et al., 2014; Giles et al., 2017). However, the proportions of N_2O emissions derived from external sources such as fertilisers or via the stimulation of N_2O production from native soil N are unclear (Schleusner et al., 2018). Further, there remains a need to determine the effects of C substrate additions on both these processes and the relative contribution of these sources to N_2O emissions.

The aim of our study was to investigate the effects of the addition of C and N substrates on the linkages between SOM priming and N_2O emissions. Specifically, our objectives were to determine the effects of different sources of C substrate (glucose, organic acids) in combination with N, as potassium nitrate, on (i) the direction and magnitude of SOM priming in the presence of N and (ii) the partitioning of N_2O emission sources. To determine these effects we applied isotopic sources of ^{13}C– and ^{15}N–labelled C and N substrates, respectively, to three soils with different properties and C concentrations. We hypothesised that (i) in the presence of N supply, SOM would be primed independently from the microbial demand for N (Chen et al., 2014; Mason-Jones et al., 2018), (ii) increased mineralisation of N due to positive priming would increase N availability from SOM and thus lead to increased SOM derived N_2O emissions, and (iii) organic acids would generate greater SOM priming than that for glucose due to the disruption of organo–mineral complexes resulting in increased N availability from SOM and, subsequently, an increased proportion of SOM derived N_2O emissions.

4.3 Materials and methods

4.3.1 Experimental design and setup

The measurements were made on soils in an experiment with a factorial design comprising three soil matric potentials (ψ, −3, −5, and −7 kPa), four added C substrates (acetate, glucose, butyrate and water as a control), three soil types and four replicates for each treatment.

Soil samples were collected to a depth of 150 mm from three grazed perennial grassland sites, all dominated by perennial ryegrass (*Lolium perenne* L.) with clover (*Trifolium repens* L.). The soils were collected from the Ashley Dene dairy farm (AD, latitude 43° 65’ S, longitude 172° 35’ E, elevation above sea level 34 m, Mottled Argillic Pallic Soil (Hewitt, 2010), Udic Ustochrept (Soil Survey Staff, 2014)), the Lincoln University long–term dairy farm (LU, 43° 65’ S,172° 48’ E, Typic Immature Pallic soil (Hewitt, 2010), Typic Haplustept (Soil Survey Staff, 2014)), and the Lincoln University Demonstration Farm (LD, 43° 65’ S,172° 44’ E, Typic Immature Pallic soil (Hewitt, 2010), Typic

Haplustept (Soil Survey Staff, 2014)). The soil samples were brought to the laboratory, air−dried at 30°C for 72 hours, sieved (≤ 2 mm; Fig. A1) with any visible plant material removed, and stored at 4°C. Soil total C and N concentrations were determined by subsampling the soil, and analysing on a CN analyser (Vario−Max CN Elemental Analyser, Elementary GmbH, Hanau, Germany). Texture analyses were performed using a laser diffraction particle analyser (Mastersizer 3000, Malvern Panalytical, U.K.). Soil pH was measured on deionised water extracts following Rowell (2014) (Table 4.1). The sieved soil was packed into stainless steel rings (73 mm internal diameter, 74 mm height) to a depth of 41 mm (Fig. A2), to achieve a bulk density (ρ_b) of 1.1 Mg m^{-3}. The bottom of each soil core was covered with a fine nylon mesh (25 µm) to prevent any soil loss. Values for soil ψ were based on those previously observed to give a range of denitrification rates (Balaine et al., 2016), and matric potentials were set by placing the cores on tension tables (Fig. A4) (Romano et al., 2002). Just prior to placing cores on the tension table, the cores were saturated with distilled water. After 4 days, during which time the soil cores' water contents had equilibrated to the desired soil ψ as determined by weighing, 1 mL of a KNO_3 ^{15}N enriched solution (300 µg N g^{-1} soil or 27.6 mg N mL^{-1}; 40 atom% excess ^{15}N, Cambridge Isotope Laboratories Inc., USA) was applied to every soil core. The small volume was used to avoid drainage and was applied evenly across the soil surface. The N substrate was added on the first day of the experiment. Three C substrates were added daily for 3 days as 0.9 mL of the substrate solution at 80 µg C g^{-1} soil (16.4 mg C mL^{-1}; 6 atom% excess ^{13}C, Cambridge Isotope Laboratories Inc., USA) by injecting the solution at 5 evenly spaced points to a depth of 20 mm using a syringe. Tension tables were maintained in a room with an average temperature of 20°C.

The C substrates selected were glucose, acetate, and butyrate along with a control (water). Glucose was selected because it is used commonly as a C source for SOM priming studies (Kuzyakov et al., 2000; Yuan et al., 2018) and to determine C substrate limitation in denitrification experiments (Morley et al., 2014). Acetate, applied as sodium acetate, was selected because its effect on N_2O production from denitrification has been shown to be different from that of glucose (Morley et al., 2014). Acetate is less easily metabolised than glucose and has a stronger effect in liberating mineral−associated C compared to the effects of glucose (Keiluweit et al., 2015). Similar to acetate, butyrate has been used as a C substrate to study denitrification (Paul et al., 1989; Morley et al., 2014) and N immobilisation (Kirchmann and Lundvall, 1993). The pH of the applied solutions were 8.74 ± 0.05 (mean ± SD, n = 3), 7.04 ± 0.04, and 7.02 ± 0.03 for acetate, butyrate, and glucose, respectively and the pH of the water was 7.01 ± 0.02. In total, 144 soil cores were packed to allow a fully replicated experimental design.

4.3.2 Measurements of $^{13}CO_2$ and $^{15}N_2O$ emissions

Based on the timing of the peak N_2O emission in experiment 1 (Chapter 1), measurements of CO_2 and N_2O emissions and their relative isotopic compositions were made three days after the addition of N. Soil cores were placed into glass jars (1 L) equipped with a gas−tight lid fitted with a rubber septa, that were permanently pierced with a needle fitted to a three−way stopcock, to allow the jars to be flushed with CO_2 free air (21% O_2, 79% N_2) (Fig. A7). Preliminary tests showed that flushing for 3 minutes resulted in a CO_2 concentration of < 15 µL L^{-1}. A syringe fitted with a two−way stopcock and a 25G hypodermic needle was used to sample 10 mL of the headspace gas for determination of N_2O concentrations at 0, 30 and 60 minutes after the jar was flushed. These samples were injected into previously evacuated 6 mL Exetainer® vials (Labco Ltd., High Wycombe, UK) for analysis by gas chromatography (SRI−8610, Torrance, CA, USA equipped with a ^{63}Ni electron capture detector). Increases in N_2O concentration were used to calculate N_2O emission rates following Hutchinson and Mosier (1981). Another gas sample (12 mL) was also collected from the headspace after 20 min of incubation. This was used for determining both the ^{13}C enrichment of the CO_2 evolved and the CO_2 concentration. The ^{13}C isotopic signature of respired CO_2 ($\delta^{13}C_{sample}$) and the concentration of the CO_2 were determined using a continuous−flow isotope ratio mass spectrometer (CFIRMS, Sercon 20−20; Sercon, Chesire, UK) interfaced to a TGII cryofocusing unit (Sercon, Chesire, UK). Soil CO_2 emissions were calculated using CO_2 concentrations at 0 and 20 mins, while assuming a linear increase in concentration, according to Holland et al. (1999).

The last gas sample (12 mL) was extracted after 180 min and used for determination of the ^{15}N enrichment of the N_2O evolved. The ion currents 44, 45, and 46 for N_2O were measured with the CFIRMS interfaced to a TGII cryofocusing unit as described by Stevens et al. (1998). Ion currents were subsequently used to determine the N_2O−^{15}N enrichment following Stevens et al. (1998).

4.3.3 Data analyses

The proportion of respired CO_2 derived from the decomposition of native SOM (fC_{som}) was calculated for all treatments using a two−source mixing model described by Kuzyakov and Cheng (2004) as

$$fC_{som} = 1 - ((\delta^{13}C_{sample} - \delta^{13}C_w)/(\delta^{13}C_{substrate} - \delta^{13}C_w)) \quad (4.1)$$

where $\delta^{13}C_w$ is the $\delta^{13}C$ value of CO_2 respired from control cores (with water addition), $\delta^{13}C_{sample}$ is the $\delta^{13}C$ value of CO_2 respired from soils with added C substrates, and $\delta^{13}C_{substrate}$ is the $\delta^{13}C$ value of the C substrate applied.

The C priming (P_C) (Table 4.2) and relative C priming, P_C expressed as a percentage of CO_2 emissions from the control treatment ($P_{C,r}$), were calculated following (Jia et al., 2017) where

$$P_C = (fC_{som} \times C_{soil}) - C_w \quad (4.2)$$

$$P_{C,r} = (P_C/C_w) \times 100 \quad (4.3)$$

where C_{soil} represents the CO_2 emissions from soils treated with substrates and C_w is the CO_2 emissions from the control (water) treatment.

The proportion of N_2O evolved from the N fertiliser (fN_F) was determined by taking the ratio of 'moles of ^{15}N enriched N_2O evolved' to the 'total moles of N_2O evolved' (enriched plus unenriched) and expressing this as a percentage (Buckthought et al., 2015). Then, the SOM derived N_2O emissions (N_{som}) were calculated as

$$N_{som} = (1 - fN_F) \times N_{total} \quad (4.4)$$

where N_{total} represents the total N_2O emissions from soils.

The fertiliser N derived N_2O emissions (N_F) was calculated from the difference between N_{total} and N_{som}.

The effects of adding substrates on C_{soil}, P_C, $P_{C,r}$, N_{total}, and soil pH were tested for significance using the Kruskal–Wallis test. The effects of soil type, C substrate, and their interactions on C_{soil} and N_{total}, P_C and N_{som} were tested using an ANOVA in the 'agricolae' package of R version 1.3.1 (De Mendiburu, 2014). The results from three levels of soil ψ were combined as soil ψ had no significant effects on the variables listed above.

4.4 Results

4.4.1 Carbon substrate effects on soil pH

Soil pH varied with substrate addition (Fig. 4.1). Addition of acetate resulted in the highest soil pH (6.3 − 7.6), followed by soil with added glucose (5.8 − 6.9), and soils with added butyrate resulted in the lowest soil pH (5.6 − 6.2) (Fig. 4.1). There was no effect of soil type on soil pH (P>0.05).

Table 4.1. Soil physical and chemical properties for the soils at Ashley Dene dairy farm (AD), Lincoln University dairy farm (LU), and Lincoln University Demonstration Farm (LD). Data shown are mean ± SD, n=3. Significance levels between soils (*P*<0.05) are denoted by a different letter.

Soil	Soil Chemistry				Soil Texture		
	Organic C ($g\ kg^{-1}$)	Total N ($g\ kg^{-1}$)	C:N ratio	pH	Clay (%) (< 2 µm)	Silt (%) (2−63 µm)	Sand (%) (> 63 µm)
AD	32.3 ± 0.4 b	3.3 ± 0.0 b	9.8 ± 0.2 bc	6.2 ± 0.3 a	12	46	42
LU	46.6 ± 1.0 a	4.5 ± 0.2 a	10.4 ± 0.5 ab	6.0 ± 0.1 a	16	48	36
LD	45.3 ± 1.7 a	4.8 ± 0.2 a	9.5 ± 0.4 b	5.8 ± 0.2 a	17	46	37

Table 4.2 Table of abbreviations.

Abbr.	Description
C_{soil}	CO_2 emissions from soils treated with carbon substrates
P_C	Carbon priming effect
$P_{C,r}$	Relative carbon priming effect as a percentage of the effect for soils with no added carbon P_C
N_{total}	Total N_2O emissions
N_{som}	N_2O emissions derived from SOM nitrogen
N_F	N_2O emissions derived from added nitrogen fertiliser

4.4.2 Carbon substrates, CO_2 emissions and the priming effect

Addition of substrate resulted in greater increases in C_{soil} than those from the addition of water alone (0.20 ± 0.04 µmol $m^{-2}\ s^{-1}$, P<0.05). Values of C_{soil} were significantly higher in LU and LD soils amended with glucose (0.73 ± 0.13 µmol $m^{-2}\ s^{-1}$) (Fig. 4.2) than those with added butyrate (0.61 ± 0.11 µmol $m^{-2}\ s^{-1}$). C_{soil} was significantly higher in the AD soil amended with glucose (0.73 ± 0.08 µmol $m^{-2}\ s^{-1}$) (Fig. 4.2) than when acetate was added (0.63 ± 0.05 µmol $m^{-2}\ s^{-1}$). Soil types had no significant effects on C_{soil} (Table 4.3).

Soil type and C substrate affected P_C, but there were no interactive effects (Table 4.4, P<0.05). Values of P_C were positive in soils treated with acetate (0.07 ± 0.09 µmol $m^{-2}\ s^{-1}$), but there was no significant effect of added glucose on priming (0.02 ± 0.10 µmol $m^{-2}\ s^{-1}$), and negative priming occurred in soils with added butyrate (-0.09 ± 0.05 µmol $m^{-2}\ s^{-1}$) (Fig. 4.2). Acetate addition resulted in significantly higher values of P_C than those with glucose addition only in the AD and LU soils.

Averaged across all C substrate treatments, values of P_C for LD (0.02 ± 0.11 µmol m^{-2} s^{-1}) and LU (0.02 ± 0.12 µmol m^{-2} s^{-1}) soils were significantly higher than those for the AD soil (-0.04 ± 0.08 µmol m^{-2} s^{-1}) (Fig. 4.2).

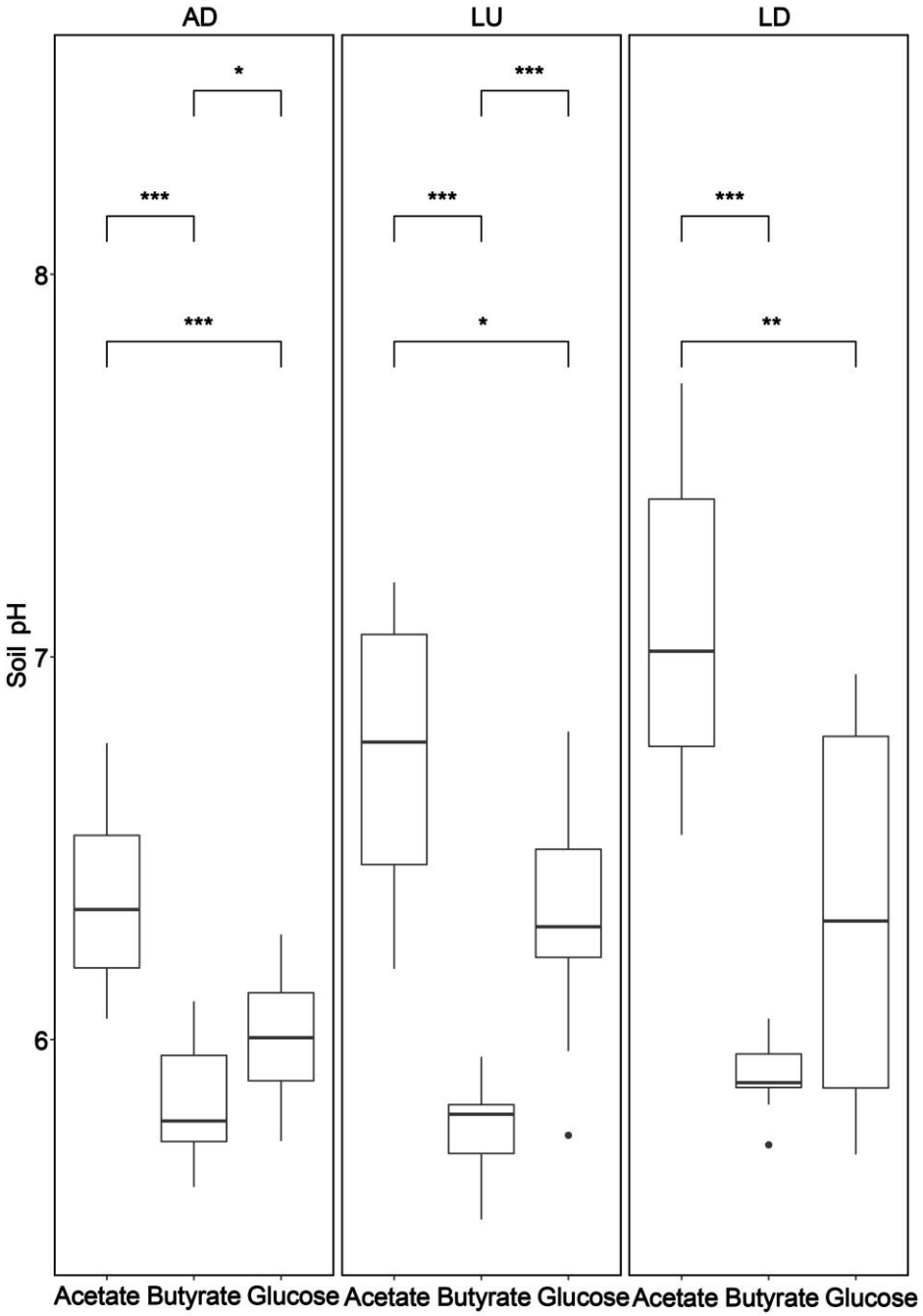

Fig. 4.1 The effects of carbon substrate addition on soil pH. Soils were sampled from three sites: Ashley Dene dairy farm (AD), Lincoln University dairy farm (LU), and Lincoln University Demonstration Farm (LD). Lower and upper whiskers are 25th and 75th percentiles, and lines within a box are median values. n=9. The Kruskal–Wallis test was conducted, * indicates significant (*P*<0.001), ** indicates significant (*P*<0.01).**

The pattern was identical when priming was expressed as a percentage (Fig. 4.2). $P_{C,r}$ from soils with added acetate (36 ± 41%) (Fig. 4.2) was significantly higher than the value for the glucose treatment (4.2 ± 46%) only for AD and LU soils, and the effects were significantly higher than the values for the butyrate treatment (-48 ± 29%) across all soils. Across all C substrates, $P_{C,r}$ for LD (11 ± 47%) and LU (8 ± 56%) soils were significantly higher than the value for the AD soil (-27 ± 48%) (Fig. 4.2).

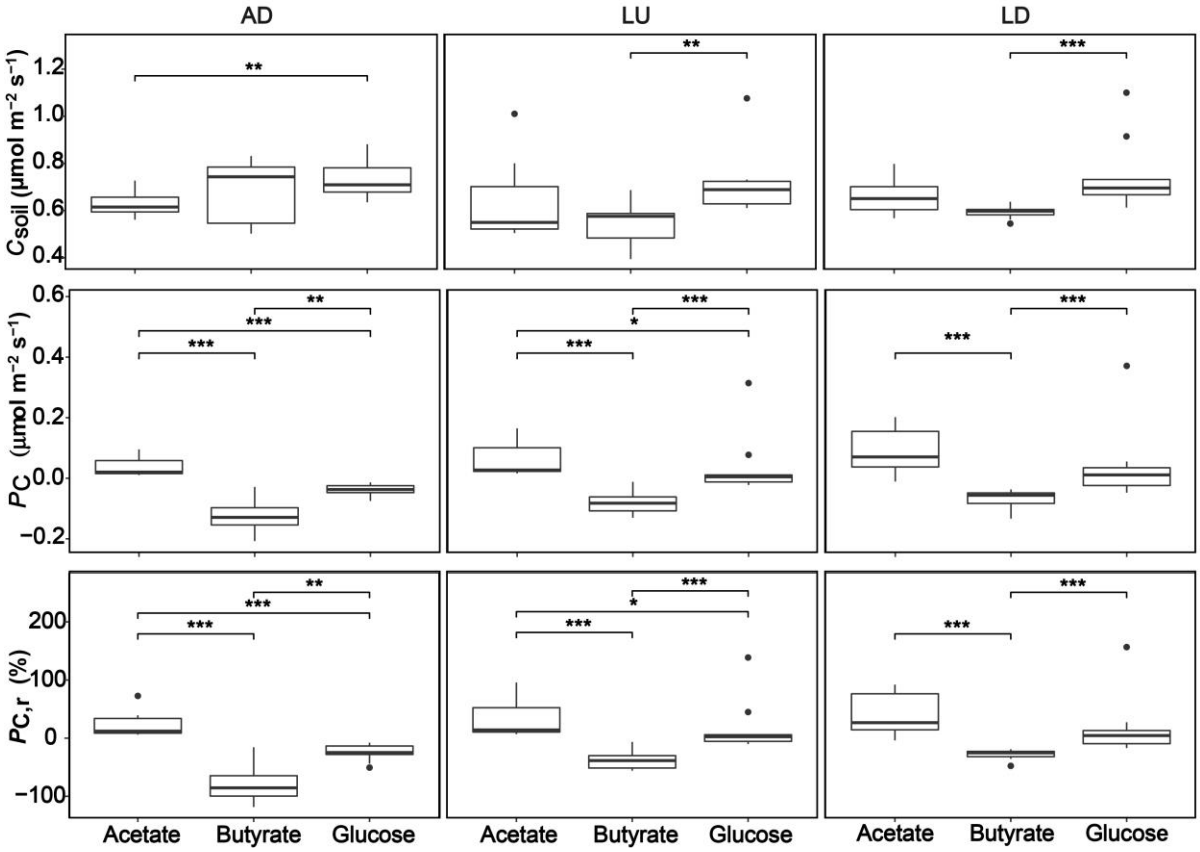

Fig. 4.2 The effects of carbon substrate addition on C_{soil}, P_C and $P_{C,r}$. Soils were treated with acetate, butyrate, and glucose. Soils were sampled from Ashley Dene dairy farm (AD), Lincoln University dairy farm (LU), and Lincoln University Demonstration Farm (LD). Lower and upper whiskers are 25th and 75th percentiles, and lines within each box are median values. n=9. The Kruskal–Wallis test shows the significance of the differences between substrates for the same soil type with *** P<0.001, ** P<0.01 and, * P<0.05. For definitions of the symbols, refer to Table 4.2.

4.4.3 Nitrogen input and N_2O emissions

Both soil type and C substrate affected N_{total} (Table 4.3) (P<0.05). Additions of substrates strongly induced SOM derived N_2O emissions from LU and LD soils (20.7 ± 8.2 mg N m^{-2} h^{-1}) (Fig. 4.3) compared with values for the control treatments (9.6 ± 1.1 mg N m^{-2} h^{-1}) (P<0.05). Values of N_{total} from LU and LD soils were significantly higher in soils amended with glucose (21.4 ± 12.1 mg N m^{-2}

h^{-1}) (Fig. 4.3) than those for soils with added acetate (10.9 ± 6.5 mg N m^{-2} h^{-1}) or butyrate (11.0 ± 6.6 mg N m^{-2} h^{-1}). Across all C substrates, N_{total} from LU (18.0 ± 8.0 mg N m^{-2} h^{-1}) and LD (21.7 ± 6.5 mg N m^{-2} h^{-1}) soils were significantly higher than that for the AD soil (3.6 ± 3.3 mg N m^{-2} h^{-1}).

Table 4.3 The effects of soil type and carbon substrate on soil carbon dioxide emissions (Cs_{oil}) and total soil N_2O emissions (N_{total}). F refers to the ratio of the variance of the group means to that of the pooled within–group variance, df refers to the degrees of freedom and *P*–values <0.05 are shown in bold type.

	C_{soil}			N_{total}		
	df	F	*P*	df	F	*P*
Soil	2	1.3	0.3	2	337.1	**<0.001**
Substrate	2	8.2	**<0.001**	2	133.1	**<0.001**
Soil: Substrate	4	1.2	0.3	4	19.2	**<0.001**

Table 4.4 The effects of soil type, carbon substrate on carbon priming (P_C) and SOM derived N_2O emissions (N_{som}). F refers to the ratio of the variance of the group means to that of the pooled within–group variance, df refers to the degrees of freedom, *P*–values <0.05 are shown in bold type.

	P_C			N_{som}		
	df	F	*P*	df	F	*P*
Soil	2	5.7	**0.005**	2	8.9	**<0.001**
Substrate	2	29.9	**<0.001**	2	17.0	**<0.001**
Soil: Substrate	4	0.1	0.9	4	4.8	**0.002**

Both soil type and C substrates affected N_{som} (Table 4.4) (P<0.05). With the exception of AD and LU soils with added glucose (Fig. 4.3), the source of the N_2O emissions was dominantly from fertiliser N, with values ranging from (18.2 ± 12.8%) to (55.9 ± 12.3%). With the exception of the LD soil, mean values of N_{som} were significantly higher from soils with glucose addition (5.1 ± 3.9 mg N m^{-2} h^{-1}) compared with those receiving acetate (0.8 ± 0.9 mg N m^{-2} h^{-1}) or butyrate addition (0.5 ± 0.9 mg N m^{-2} h^{-1}). In the LD soil, N_{som} was higher from the soil with added acetate (2.9 ± 2.7 mg N m^{-2} h^{-1}) than the value for soils with added butyrate (1.2 ± 1.3 mg N m^{-2} h^{-1}, P<0.05).

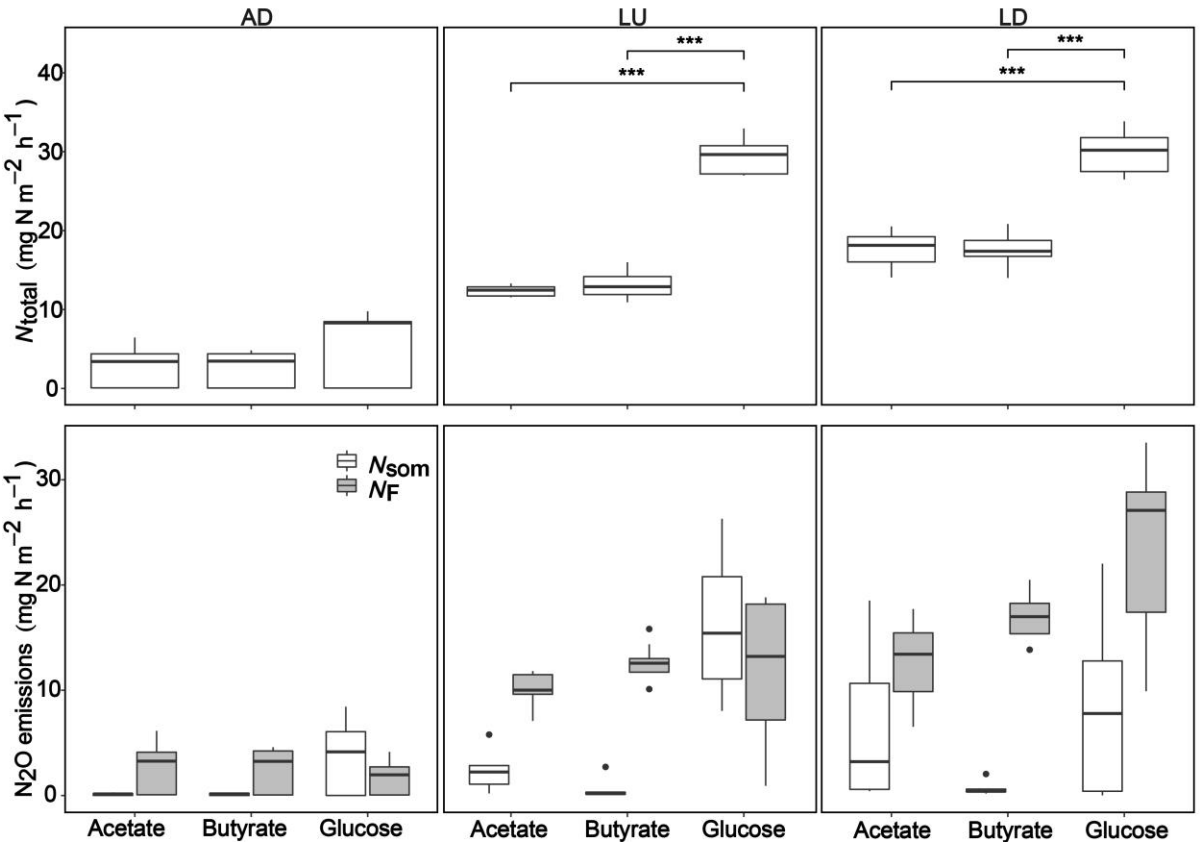

Fig. 4.3 The effects of carbon substrate addition on N_{total}, N_{som} and N_F. Soils were treated with acetate, butyrate, and glucose. Soils were sampled from Ashley Dene dairy farm (AD), Lincoln University dairy farm (LU), and Lincoln University Demonstration Farm (LD). Lower and upper whiskers are 25th and 75th percentiles, and lines within each box are median values. n=9. The Kruskal–Wallis test shows the significance of the differences between substrates for the same soil type with *** P<0.001, ** P<0.01 and, * P<0.05. For definitions of the symbols, refer to Table 4.2.

4.5 Discussion

Using stable isotopes of both C and N, our study provides new insights into determining the effects of adding C substrates to soil with respect to SOM decomposition and SOM derived N_2O emissions in the presence of N fertiliser. Addition of C substrates increased both CO_2 and N_2O emissions significantly, with the priming effects on SOM and SOM derived N_2O emissions dependent on the chemical nature of the C substrate. Addition of acetate resulted in positive priming while the addition of glucose had no significant effect on priming. However, glucose addition resulted in more SOM derived N_2O emissions than the effects of adding acetate. Butyrate caused a negative priming effect and had the lowest effect on SOM derived N_2O emissions.

4.5.1 Carbon input and priming

Relative to soils with no C addition, emissions of CO_2 from soils with added C substrates increased strongly. Increases in CO_2 emissions may be attributable to both the decomposition of the C substrates themselves and priming effects stimulating the decomposition of SOM as shown in Fig. 4.4 (Kuzyakov et al., 2000; Cheng et al., 2014; Shahbaz et al., 2018). Emissions of CO_2 from soils with added glucose were higher than those from soils treated with acetate or butyrate (Fig. 4.2). This is because glucose enters glycolysis directly and is metabolised rapidly (Gunina et al., 2014), resulting in high C use efficiency in terms of the relative partitioning of C between microbial anabolic and catabolic processes (Jones et al., 2018). Thus, glucose is recognised as providing the main source of C for a wide range of microbial communities (Paterson et al., 2007). Conversely, butyrate provides limited energy for microbial processes (Paul et al., 1989), as shown in (Fig. 4.4a) and acetate provides even less energy (Paul et al., 1989; Gunina et al., 2014).

The significantly greater priming effects induced by acetate addition, relative to glucose addition, are likely attributable to acetate's additional effects of disrupting physically protected organo−mineral complexes (Keiluweit et al., 2015; Yuan et al., 2018). Other studies have shown that acetate addition strongly stimulates microbial activity and SOM turnover in paddy soils (Wei et al., 2019). However, the effects of priming from glucose additions have been shown to be less with repeated additions than those from a single addition (Qiao et al., 2014). In our study, daily additions of glucose might have satisfied the energy requirements for microbial growth, leading to our observations of the lack of a priming effect. An alternative explanation is that added glucose increases the formation of physically protected organo−mineral complexes (Baumert et al., 2018) and thereby limits microbial and enzymatic access to the SOM (Conant et al., 2011).

Keiluweit et al. (2015) observed that priming effects are attributable to changes in mineral−SOM interactions, themselves related to changes in soil pH. The authors showed that reduced SOM decomposition in the presence of acetate was in response to an increase in soil pH and that oxalate increased SOM decomposition while reducing soil pH. On this basis, our observations of changes in soil pH (Fig. 4.1) could also explain the observed priming effects (Fig. 4.2), with the lowest soil pH from butyrate addition associated with negative priming, and the highest pH from acetate addition leading to positive priming. With decreasing pH following butyrate addition compared to acetate addition (Fig. 4.1), the equilibrium for the reactions driving the formation of mineral−SOM complexes would have resulted in a decrease in soluble SOM, therefore reducing available substrate for microbial decomposition, resulting in similar effects from acetate and oxalate addition, as reported by Keiluweit et al. (2015).

4.5.2 Nitrogen inputs and priming effects

We showed that, generally, addition of glucose resulted in increased SOM derived N_2O emissions, probably resulting from increased denitrification under anaerobic conditions with sufficient availability of C substrates (Petersen et al., 2008; Friedl et al., 2016). Uchida et al. (2011) showed that plant−derived C inputs promoted the activity of N_2O producing microbes due to enhanced anaerobic conditions. The rate of microbial fatty acid synthesis also increases with the addition of glycolysis−derived substrates such as glucose (Murray et al., 2004; Gunina et al., 2014). Hence, glucose is likely to favour the formation of anoxic microsites and thus promote N_2O emissions (Morley and Baggs, 2010). The SOM derived N_2O emissions were higher for the more finely textured soils, LU and LD, than those for the AD soil (Fig. 4.3). Although we showed no significant effects of differences in soil water content, this is possibly due to more restricted oxygen diffusion that would result in more anaerobic microsites and increased rates of denitrification (Pelster et al., 2012; Balaine et al., 2016; Chamindu Deepagoda et al., 2019). In contrast, lower SOM derived N_2O emissions from the AD soil were likely a consequence of the coarser soil texture with reduced ability to hold water, leading to high relative gas diffusivity and oxygen supply and reduced rates of denitrification (Balaine et al., 2016; Chamindu Deepagoda et al., 2019).

Using stable isotopes, we were able to partition total emitted N_2O into two sources: the external fertiliser N or the native soil N. Decomposition of SOM provides a source of ammonium (NH_4^+) and, when this is oxidised to nitrate (NO_3^-), this serves as a substrate for denitrification. In our study, we assume that most of the N_2O emissions were derived from denitrification because the matric potentials were higher than −7 kPa (Balaine et al., 2016). Denitrification can result from the activities of heterotrophic microorganisms (Butterbach-Bahl et al., 2013), so denitrification consumes energy that is associated with the bioavailability of C substrates that are, in turn, modified by priming effects (Sørensen, 1998; Gunina et al., 2014; Mason-Jones et al., 2018). Thus, the N_2O emissions derived from SOM can be partly explained by C substrate−induced priming effects. Results showed that the N_2O emissions from SOM, with added C substrates, were derived dominantly from fertiliser N (up to 68%), consistent with the study by Schleusner et al. (2018), who found >80% of the N_2O emissions derived from added N, and Buckthought et al. (2015) who found up to 75% was derived from added urea N under field conditions.

4.5.3 Linkage between carbon priming effects and soil derived N_2O emissions

The addition of C substrate affected the rate of decomposition of SOM and total N_2O emissions in the presence of added N (Table 4.4, Fig. 4.4). Added C substrate provides a direct source of energy to satisfy energy demands of denitrifiers and, in the presence of N, this results in an increase in N_2O emissions (Morley and Baggs, 2010; Giles et al., 2017). Thus, glucose, a highly energetic substrate for soil microbes, resulted in higher SOM derived N_2O emissions.

Substrate addition also changes the rate of microbial decomposition of SOM, and this may increase or decrease native substrate availability and subsequent energy for denitrifying activity (Qiao et al., 2016; Mason-Jones et al., 2018). The rate of SOM decomposition may also alter soil N mineralisation rates (Pelster et al., 2012; Buckthought et al., 2015; Jones et al., 2018). Priming effects can therefore modify both C and N availability for denitrifiers. With less energy available from added acetate and butyrate compared with that from glucose (Paul et al., 1989), denitrifiers in soils with added butyrate and acetate would have obtained more energy from SOM decomposition. In the presence of butyrate and acetate, we would therefore have expected a relationship between SOM priming and SOM derived N_2O emissions.

Consistent with the anticipated effects of butyrate discussed above, we anticipate that the addition of butyrate would result in negative priming effects and relatively low N_2O emissions derived from SOM. On the other hand, soils with added acetate and positive priming effects would be expected to lead to a higher proportion of N_2O emissions derived from SOM. However, we did not detect a direct relationship between priming effects and N_2O emissions derived from SOM. A general interpretation of these findings is that denitrifiers become energy limited only when negative priming effects in the presence of butyrate are strongest, while acetate provides sufficient energy from the SOM decomposition and acetate itself to sustain the energy requirements of denitrifiers. Adding glucose, however, leads to the availability of energy being independent from that derived from priming effects, and results in high SOM derived N_2O emissions in the presence of non−limiting N supply.

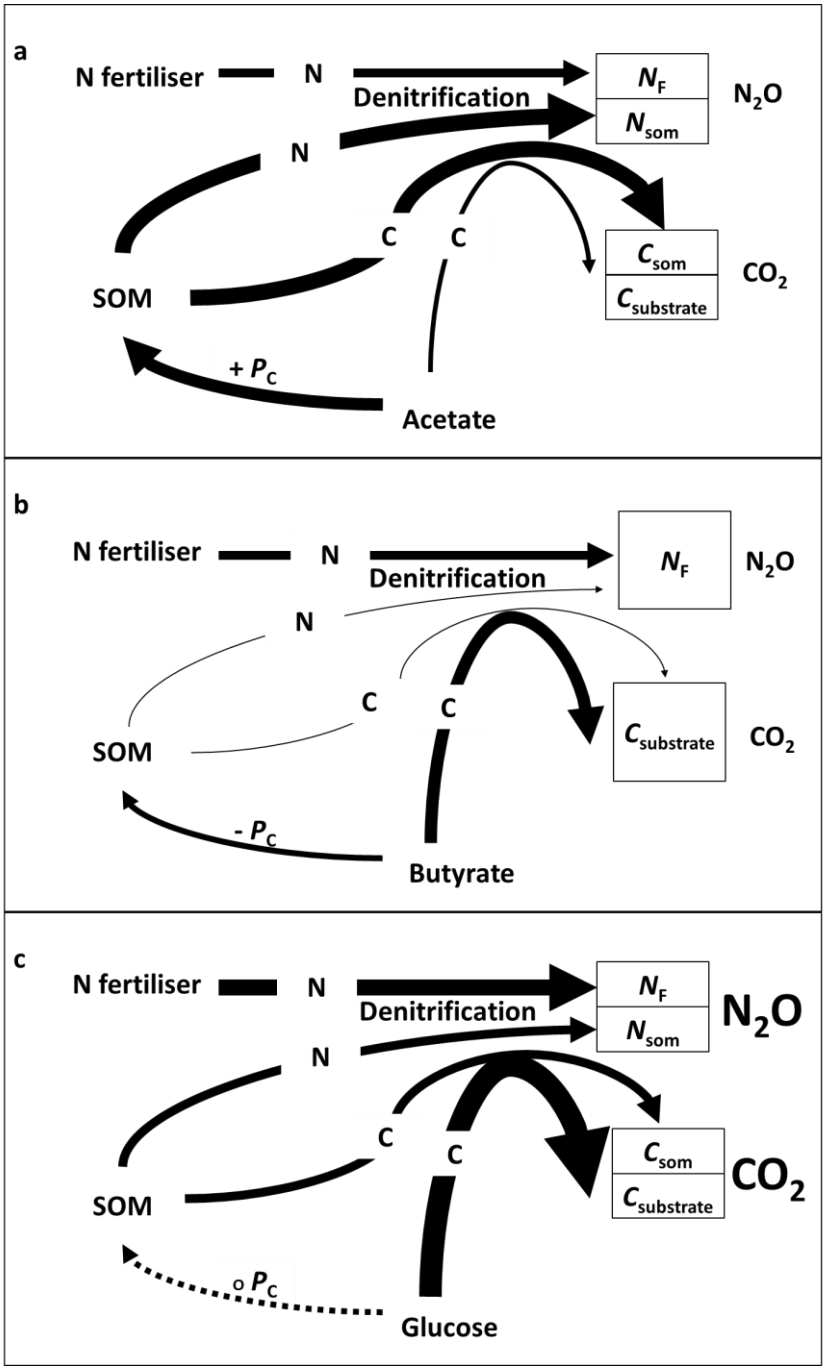

Fig. 4.4 Conceptual diagram of nitrous oxide and carbon dioxide emissions as affected by substrate source. The thickness of the line highlights the strength of the emission. The box partitioning reflects the relative sources of carbon dioxide (CO_2) soil organic matter (C_{som}) vs. substrate ($C_{substrate}$); nitrous oxide (N_2O) from soil organic matter (N_{som}) vs. nitrogen fertiliser (N_F). Symbols + P_C, − P_C, and o P_C represent positive, negative, and neutral priming effects, respectively.

Relationships between C and N supply, priming and the sources of N for N_2O production were developed using our observations and the conceptual models shown in Fig. 4.4. Compared with glucose and butyrate, acetate provides the least energy for microbes (Paul et al., 1989; Gunina et al.,

2014), but results in the strongest positive priming effects due to the higher dissolution of mineral−associated C that provides additional energy for heterotrophic denitrification (Fig. 4.4a). Butyrate addition results in negative priming which limits access, by heterotrophic denitrifies, to N from SOM decomposition, so this leads to the lowest SOM derived N_2O emissions (Fig. 4.4b). However, butyrate provides more energy for heterotrophic denitrification than that of acetate (Paul et al., 1989). Our interpretation is that the energy provided to microbes by adding acetate is overall the same as that for butyrate because the lower energy from the substrate is offset by more energy from increased SOM decomposition. This leads to the same N_2O emissions, but from different sources, as access to native N is limited by the negative priming induced by butyrate. The glucose has the highest available energy (Paul et al., 1989; Gunina et al., 2014) and glucose has no effect on SOM decomposition (neutral priming), which does not limit access to native N (Fig. 4.4c). Thus, for glucose addition, a large amount of energy available for heterotrophic denitrification therefore leads to high N_2O emissions from both SOM and fertiliser N.

We therefore partly confirm our original hypothesis: in the presence of an external supply of N, negative priming limited the availability of native soil N and therefore resulted in no SOM derived N_2O emissions. However, there was not a clear relationship between the magnitude of SOM priming and SOM derived N_2O emissions. We argue that lack of such a relationship is due to additional factors regulating the activity of denitrifiers, one of which is likely to be the energy available from SOM decomposition and the decomposition of added substrates with different energy yields (Gunina et al., 2014).

4.6 Conclusions

The relatively low N_2O emissions derived from SOM associated with butyrate addition in this study were accompanied by a negative priming effect. These findings suggest that negative priming effects may limit both the pool of N available from SOM mineralisation and the energy available for denitrification, and lead to reduced N_2O emissions even in conditions of non−limiting N availability. Under the same conditions, glucose addition provided sufficient energy to increase denitrifier activity, resulting in increased N_2O emissions, both from the native N source and added N. Our study revealed a link between energy availability from substrate addition and SOM decomposition as modified by priming effects and highlights the need to consider the nature of C substrates when interpreting the interactive processes regulating SOM decomposition and SOM derived N_2O emissions.

Chapter 5 Effects of irrigation frequency on the components of ecosystem carbon balance and nitrous oxide emissions for a C_4 grassland growing in mesocosms

This chapter has been submitted to Pedosphere (manuscript ID pedos201909626).

5.1 Abstract:

Intensification of grazed grasslands following conversion from dryland to irrigated farming is a major land−use change in New Zealand. Such conversion has the potential to alter ecosystem carbon (C) cycling and affect components of the C balance that could lead to either net accumulation or loss of soil C. While there are many studies of the effects of water availability on biomass production and soil C stocks, much less is known about the effects of the frequency of water inputs on the components of the C balance. Here, the components of net ecosystem CO_2 exchange (F_N) were partitioned for a C_4 plant (Bermuda grass, *Cynodon dactylon* L.), growing in mesocosms and irrigated to return the soil water content to field capacity but with water applied at intervals of either 1, 2, or 3 days (treatments I_1, I_2, and I_3, respectively) for 12 days, after which the I_2 treatment was changed to watering every 6 days (treatment I_2/I_6), and all treatments were continued for a further 18 days. Daily measurements of evaporation were made by weighing the mesocosms and a chamber was used to measure rates of carbon dioxide (CO_2) exchange to estimate F_N, ecosystem respiration (R_E) and soil respiration (R_S), with gross C uptake by the plants (F_G) and respiration from leaves (R_L) also calculated. During the first 12 days, there were no significant differences in cumulative F_N (overall mean ± SD, 61 ± 30 g C m^{-2}, n = 4). During the subsequent 18 days, cumulative F_N decreased with decreasing irrigation frequency and increasing cumulative soil water deficit (*W*), with values of 70 ± 22, 60 ± 16, and 18 ± 12 g C m^{-2} for the I_1, I_3, and I_2/I_6 treatments, respectively. There were similar decreases in F_G, R_E and R_L with increasing *W* but differences in R_S were not significant. Use of the C_4 plant enabled partitioning of R_S into its autotrophic (R_A) and heterotrophic (R_H) components using a ^{13}C natural abundance isotopic technique, requiring destructive sampling at the end of the experiment when differences in cumulative *W* between the treatments were greatest. The value of R_H and its percentage contribution to R_S (43 ± 8, 42 ± 8, and 8 ± 5% for the I_1, I_3, and I_2/I_6 treatments, respectively) suggested that R_H remained unaffected across a wide range of *W*, then decreased under extreme *W*. There were no significant differences in aboveground biomass between the treatments and there was a decrease in nitrous oxide (N_2O) emissions with increasing *W*. These

findings suggest that, over short periods in well−drained soil, irrigation frequency could be managed to manipulate soil water deficits to reduce net belowground respiratory C losses, particularly those from the microbial decomposition of soil organic matter, with no significant effects on biomass production and N_2O emissions.

Keywords: carbon balance; ^{13}C natural abundance; irrigation frequency; nitrous oxide, soil heterotrophic respiration

5.2 Introduction

Conversion of non−irrigated grasslands to high−intensity farm systems with irrigation is a major land−use change in dryland areas of New Zealand, and is undertaken to increase feed supply during periods with low rainfall (MacLeod and Moller, 2006). For example, Condron et al. (2014) reported increases in biomass production of 44 and 70% with irrigation treatments in dry periods of 10 and 20% receiving the annual rainfall of 740 mm yr^{-1}, respectively, compared with the production at a control site with no irrigation. However, despite increased aboveground production, there is increasing evidence that irrigation in New Zealand's grazed grasslands leads to lower (Houlbrooke et al., 2008; Mudge et al., 2017) or no change (Condron et al., 2014; Kelliher et al., 2015) in soil C stocks when compared with stocks at adjacent non−irrigated sites. Conversely, in humid environments similar to those in New Zealand, the effects of irrigation on soil C stock are inconsistent (Trost et al., 2013). With the recognised need to increase soil C stocks to improve soil quality and offset greenhouse gas emissions (Rumpel et al., 2018), there is an urgent need to investigate the processes regulating inputs and losses of C in irrigated grasslands to better inform managers of practices that can be used to minimise losses.

Irrigation to increase water content in the root zone changes the rates of photosynthesis and respiration in grasslands (Scott et al., 2009; Hussain et al., 2015). Irrigation also changes the components of the C balance (Moinet et al., 2016b; Moinet et al., 2017; Whitehead et al., 2018) by modifying the microbial processes that regulate C and N cycling (Entry et al., 2008; Trost et al., 2013; Karlowsky et al., 2018).

The magnitude of the proportional changes in ecosystem respiration (R_E) and photosynthesis in response to changes in soil water availability may not be equal, leading to changes in net ecosystem CO_2 exchange (F_N). Other studies using eddy covariance estimates of net CO_2 exchange have shown that gross photosynthesis (F_G) is more sensitive to water availability than R_E (Schwalm et al., 2010; Hunt et al., 2016). This may, in part, be due to differences in the allocation of photosynthates to roots and shoots subsequently affecting the relative proportions of aboveground respiration from

leaves (R_L) and root respiration (R_A), so the ratio of R_L to R_A will not be linear with changes in soil water availability (Mokany et al., 2006). Water availability also has proportionally different effects on autotrophic respiration from roots (R_A) and heterotrophic respiration from the decomposition of soil organic matter (R_H) (Moinet et al., 2016a; Zhou et al., 2016; Zhang et al., 2019), the two components that comprise soil respiration (R_S), because R_A is strongly dependent on the supply of carbohydrates from photosynthesis (Huxman et al., 2004). A meta−analysis of global data on the effects of drought and irrigation on C balance components showed that drought−induced soil water deficits resulted in decreases in aboveground and belowground net primary production, plant C pools, R_S and its components while, conversely, irrigation induced increases in these variables (Zhou et al., 2016). In a review across a range of crops and soil types, Sadras and Milroy (1996) found that typical responses of gas exchange rates to plant available soil water could be described with two straight lines that intersect at a threshold value. In terms of soil water deficit, the threshold value indicates a value below which measured variables remain constant and above which measured variables decline. The threshold value is appropriate for evaluating physiological effects (Marshall et al., 2013) and the response can be fitted using a broken−stick linear regression model (Davidson et al., 1998; Wei et al., 2010).

Emissions of nitrous oxide (N_2O) from grassland increase with the addition of N fertiliser and the deposition of ruminant urine (Scheer et al., 2008; Owens et al., 2016). Studies at irrigated sites have demonstrated pulses of N_2O emissions following N fertiliser application, which can account for up to 90% of annual N_2O losses when denitrification occurs in soils with high water content (Scheer et al., 2008; Mumford et al., 2019). Irrigation practices displace air from soil pores (Owens et al., 2016), creating aerobic conditions required for the activity of denitrifiers (Tiedje, 1988). As soils dry and become aerobic, N_2O emissions gradually decline (Balaine et al., 2016). However, while many studies have established relationships between the intensity of irrigation and N_2O emissions (Scheer et al., 2008; Owens et al., 2016; Carlton et al., 2018; Mumford et al., 2019), there has been less emphasis on the effects of the frequency of water application.

Since the interacting microbial processes that regulate both C and N cycling are sensitive to soil water content, there is an opportunity to schedule the frequency of irrigation to minimise both the rate of soil organic matter (SOM) decomposition (Metherell, 2003) and N_2O emissions (Scheer et al., 2008; Mumford et al., 2019). While increased water availability under irrigation is likely to lead to increased biomass production, and a higher input of C into the soil (Kochsiek et al., 2009; Whitehead et al., 2018), a higher soil water content also enhances soil microbial activity (Fuchslueger et al., 2014) and increases rates of decomposition (Schipper et al., 2013; Condron et al., 2014), especially during periods when soil temperature is seasonally high (Mudge et al., 2017).

This study investigated the effects of irrigation frequency on the C balance and N_2O emissions using a model grassland system growing in mesocosms under controlled conditions. Estimates of ecosystem C balance components for the plants and soil were made from measurements of CO_2 exchange over a period of 30 days. The use of the C_4 Bermuda grass (*Cynodon dactylon* L.) allowed partitioning of belowground respiration sources from measurements of the natural abundance of the $\delta^{13}C$ stable isotope. Measurements of N_2O emissions from soil in the same mesocosms were made. It was hypothesised that increasing cumulative soil water deficit (*W*) resulting from decreasing irrigation frequency would lead to (i) decreased F_N because of both reduced photosynthesis and aboveground plant respiration, (ii) increased ratio of root to heterotrophic belowground respiration (R_A/R_H) comprising R_S and (iii) decreased soil N_2O emissions because of increased soil aerobic conditions.

5.3 Materials and methods

5.3.1 Mesocosms preparation and experimental design

Topsoil (0–150 mm depth) was collected from a grazed grassland site dominated by perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) from the Lincoln University Demonstration Farm (lat. 43° 65' S, long. 172° 48' E, elevation above sea level 34 m). The soil was classified as a Typic Immature Pallic soil (Hewitt, 2010), Typic Haplustept (Soil Survey Staff, 2014). The soil was mixed and sieved (≤ 4 mm; Fig. A1), with all visible plant material removed, prior to storage at 4°C. Soil total C and N concentrations were determined on soil subsamples, with a CN Elemental Analyser (Model Vario−Max, Elementary GmbH, Hanau, Germany). Soil pH was measured using a deionised water extraction method following Rowell (2014) (Table 5.1). The sieved soil was packed into 12 PVC cylinders (200 mm internal diameter, 300 mm deep) to a depth of 270 mm, to achieve a bulk density (ρ_d) of 0.9 Mg m^{-3}. The bottom of each mesocosm was covered with a fine nylon mesh (25 µm) to prevent any soil loss. A PVC collar (100 mm internal diameter, 70 mm deep) with a removable lid was inserted in the centre of each mesocosm to a depth of 30 mm to allow the collection of gases for measurements of R_S and N_2O emissions (Fig. A3).

In order to use a natural abundance ^{13}C isotopic approach to partition the sources of R_S, the C_4 Bermuda grass (*Cynodon dactylon* L.) was grown in the mesocosms. Fifteen Bermuda grass seeds (10 g m^{-2}, PGG Wrightson Seeds, Christchurch, NZ) were sown in the annulus outside each PVC collar. The mesocosms were then placed in a growth cabinet (Model HGC 1514, Weiss Gallenkamp, UK) set at constant conditions of air temperature 25°C, photoperiod 16 hours at an irradiance (400–700 nm)

of 600–650 µmol m^{-2} s^{-1}, relative humidity 70% and the grass was left to grow with a plentiful water supply for 12 weeks.

Table 5.1 Soil physical and chemical properties. Data shown are mean ± SD, n=3.

Items	Unit	
Total carbon concentration	mg kg^{-1}	4.53 ± 0.20
Total nitrogen concentration	mg kg^{-1}	0.48 ± 0.02
Clay content (< 2 µm)	%	17
Silt content (2−63 µm)	%	46
Sand content (> 63 µm)	%	37
Water holding capacity*	mm	134 ± 3
pH		5.8

*Depth of the mesocosm is 270 mm.

At the start of the experimental period, the grass was clipped to a height of 30 mm above the soil surface, ammonium sulphate solution (1 M) was applied to supply N equivalent to 50 kg N ha^{-1} and, for a further six days, the mesocosms were watered to ensure the soil water content remained at field capacity. Subsequently, the mesocosms were weighed to measure water loss from evaporation and this amount of water was applied to return the soil water content to field capacity (mean ± SD, 134 ± 3 mm H_2O), but at four different frequencies that comprised the experimental treatments. There were four replicate mesocosms for each treatment. In the first 12 days, the frequency of application was either each day (treatment I_1), every two days (I_2), or every three days (I_3). After day 12 there were no significant differences in the components of the C balance so the I_2 treatment was modified to a lower irrigation frequency of every 6 days (treatment I_6). The mesocosms were placed on saucers to allow the plants to access all water supplied with no losses from drainage.

Soil volumetric water content (θ_v) was measured every 15 min in each mesocosm using sensors (CS 616 Reflectometer, Campbell Scientific, Logan, UT) attached to a data logger and multiplexer (Model CR 1000, AM416, Campbell Scientific, Logan, UT, USA) installed in two replicates for each treatment diagonally across the soil profile. Water−filled pore space (WFPS) was calculated from θ_v (WFPS = θ_v /(1− ρ_b/ρ_p), where the soil bulk density (ρ_b) was 0.9 Mg m^{-3}, and particle density (ρ_p) was assumed to be 2.65 Mg m^{-3} for all soils (Nimmo, 2004). Daily soil water deficit was calculated from the difference between the value of θ_v for soil at field capacity and the actual value of θ_v, converted into a water depth from the volume of soil in each mesocosm and summed to give values of cumulative water deficit (*W*).

At the end of the experiment, the aboveground biomass was cut to 30 mm above the soil surface, dried at 60°C, and weighed to determine the total dry matter production during the experimental period. For further laboratory analysis, the material was ground using a ball mill.

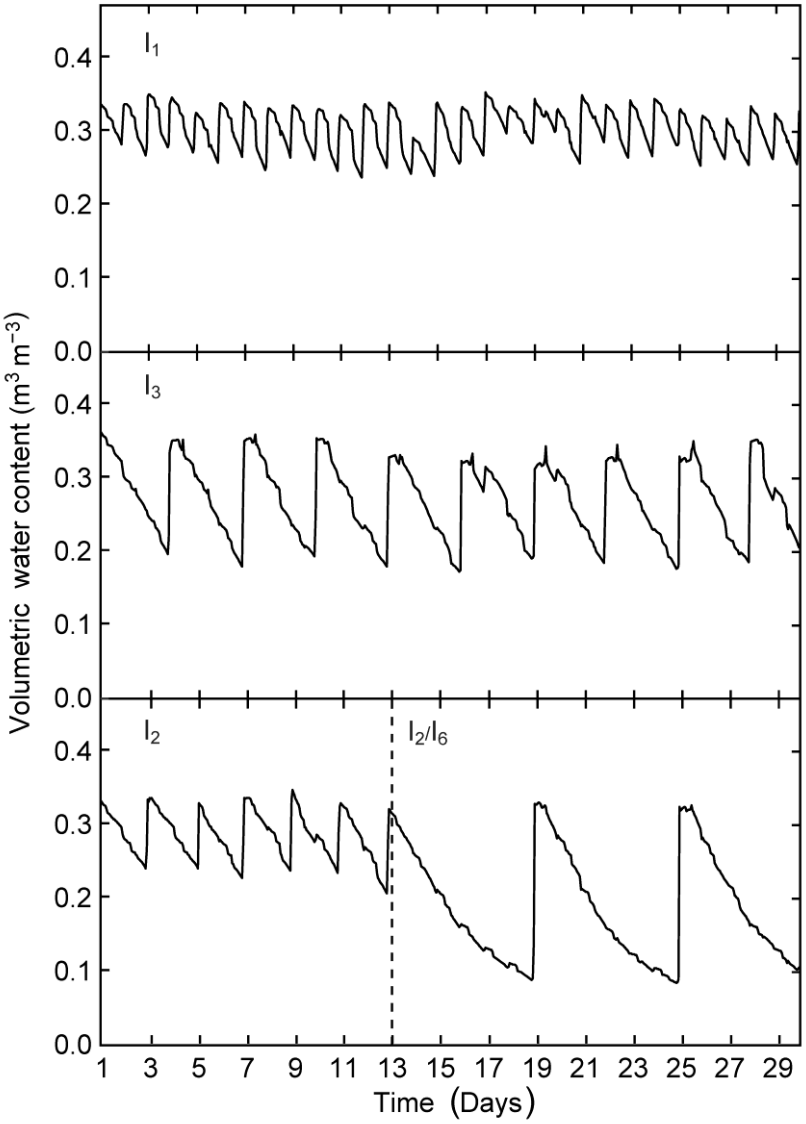

Fig. 5.1 Soil volumetric water content at depths from 50 to 250 mm for the irrigation treatments over the 30 days. The irrigation frequencies are every day, every two, three days and every six days (I_1, I_2, I_3 and I_2/I_6, respectively, n=4. On day 13 the I_2 treatment was changed to the I_6 treatment as indicated by the vertical line.

5.3.2 Measurements of net ecosystem CO_2 exchange, soil respiration and N_2O emissions

Ecosystem net CO_2 exchange (F_N) is the difference between gross photosynthesis (F_G) and ecosystem respiration from plants and soil (R_E) such that

$$F_N = F_G - R_E \quad (5.1)$$

R_E is comprised of aboveground plant respiration (R_L) and soil respiration (R_S) where

$$R_E = R_L + R_S \quad (5.2)$$

and R_S is comprised of autotrophic root respiration (R_A) and microbial decomposition of SOM (heterotrophic respiration R_H) so

$$R_S = R_A + R_H \quad (5.3)$$

Net ecosystem CO_2 exchange was estimated on each mesocosm under full irradiance (400−700 nm) of 600−650 µmol m^{-2} s^{-1} by measuring the rate of change of CO_2 partial pressure in a purpose−built cylindrical polycarbonate chamber (200 mm diameter and 210 mm height) placed on the top of each mesocosm (Fig. A6a) over a period of 2 min as described by Moinet et al. (2016b). We adopt the terminology that positive values of F_N indicate net uptake of CO_2. Subsequently, the measurement was repeated to estimate R_E by excluding light using a dark cloth placed over the mesocosm and chamber (Fig. A6b). R_S was then measured by placing a chamber from an automatic soil respiration system (Model LI−8100, LI−COR Inc., Lincoln, Nebraska, USA) on the central collar in each mesocosm (Fig. A6c). In addition, F_G and R_L were also calculated according to equations 1 and 2. Subsequently, the central collar was sealed with a gas−tight lid fitted with a two−way stopcock, and a 25G hypodermic needle was used to remove gas samples (10 mL) for measurements of N_2O partial pressure at 0, 30 and 60 minute intervals after the lid was sealed (Fig. A6d). These samples were injected into previously evacuated 6 mL Exetainer® vials (Labco Ltd., High Wycombe, UK) for analysis by gas chromatography (SRI−8610, Torrance, CA) equipped with a ^{63}Ni electron capture detector. The rate of increase in N_2O partial pressure was used to calculate N_2O emissions following Hutchinson and Mosier (1981). Measurements of net ecosystem CO_2 exchange, R_S and N_2O emission were made each day over 30 days and the values integrated to give cumulative values.

5.3.3 Partitioning the sources of soil respiration

At the end of the experimental period when differences in *W* between the treatments were greatest, the natural abundance $\delta^{13}C$ isotope technique was used to partition R_S into R_H and R_A. The technique requires the measurement of ^{13}C isotopic signatures of the CO_2 respired from the undisturbed soil ($\delta^{13}CR_S$), and the isotopic signature of roots ($\delta^{13}CR_A$) and root−free soils ($\delta^{13}CR_H$). Measurements of $\delta^{13}CR_S$, from each treatment, were made by collecting air respired from the soil surface using a partially automated open−chamber system described in detail by Midwood et al. (2008). The chambers were placed on the central collar in each mesocosm and CO_2 free air was supplied at a variable rate to maintain the CO_2 partial pressure inside the chamber at 500 µmol mol^{-1} (Fig. A8a). After an equilibration period of about 90 minutes, 500 mL of respired air was collected into gas−tight sample bags (Tedlar® Keika Ventures, Chapel Hill, NC, USA) that were flushed twice with CO_2 free air and evacuated before use (Fig. A8b). The gas was analysed for $\delta^{13}C$ values using a cavity ringdown spectrometer (model G2121−I, Picarro Inc., Santa Clara, CA, USA). The $\delta^{13}C$ signature for the reference gas was calibrated using Pee−Dee−Belemnite (PDB) as the certified standard.

Immediately after the measurements of $\delta^{13}CR_S$, the mesocosms were sampled destructively for collection of roots and soil samples (Fig. A8d). Plant roots were separated from the soil, washed with deionized water and dried in an oven at 65°C. One composite soil sample was taken from each mesocosm and a sub−sample was freed from root material and dried in an oven at 105°C. All oven−dried materials were ground in a ball mill, and $\delta^{13}C$ composition was measured using a continuous−flow isotope ratio mass spectrometer (Model CFIRMS, Sercon 20−22; Sercon, Cheshire, UK) interfaced with a TGII cryofocusing unit (Sercon, Cheshire, UK) to determine the values of $\delta^{13}CR_A$ and $\delta^{13}CR_H$.

The proportion of respiration derived from heterotrophic respiration, fR_H, and the rate of heterotrophic respiration, R_H, were calculated using a mass balance approach (Millard et al., 2010; Moinet et al., 2016a; Moinet et al., 2016b) from

$$fR_H = 1 - (\delta^{13}CR_S - \delta^{13}CR_H) / (\delta^{13}CR_A - \delta^{13}CR_H) \quad (5.4)$$

$$R_H = fR_H \times R_S \quad (5.5)$$

5.3.4 Data analyses

Significance of cumulative values of F_N, F_G, R_E, R_S, R_L, N_2O emissions and values of R_A and R_H were tested in a one−way analysis of variance (ANOVA), then differences between treatments were compared using Tukey's *HSD* test in the 'agricolae' package of R (De Mendiburu, 2014). Relationships

between F_N, F_G, and R_E with increasing W were fitted using a broken−stick linear regression model with a critical limiting value for W below which the variables remained constant and above which the values began to decrease using the 'segmented' package for R (Muggeo, 2008).

5.4 Results

5.4.1 Aboveground biomass production

There were no significant differences in aboveground dry matter biomass production with irrigation frequency over the 30 days with mean ± SD values of 660.4 ± 71.9, 598.9 ± 18.0 and 571.7 ± 34.7 g m $^{-2}$ for the I_1, I_3 and the combined I_2/I_6 treatments, respectively.

5.4.2 Responses of respiration components to cumulative water deficit

Preliminary analysis of the data indicated that the response of the C balance components to increasing W, using the 'broken stick' model, showed that the threshold values above which the components remained constant were 29, 28, and 29 mm for F_N, F_G, and R_E, respectively (Fig. 5.2a). As W increased beyond these critical values, F_N declined from 5 to –9 µmol m^{-2} s^{-1} with 31% of the variation explained (P<0.001, Fig. 5.2). Cumulative values of F_N of 69 ± 33, 57 ± 31, and 57 ± 27 g C m^{-2} for the I_1, I_2, and I_3 treatments, respectively, were not significantly different during first 12 days but were different with values of 70 ± 22, 60 ± 16, and 18 ± 12 g C m^{-2} for the I_1, I_3, and I_2/I_6, treatments, respectively, for the subsequent 18 days (Table 5.2). Values of F_G also declined with increasing W, with the model explaining 35% of the variation (P<0.001, Fig. 5.2b). Cumulative values of F_G were not significantly different up to day 12 (365 ± 50, 345 ± 62, and 329 ± 40 g C m^{-2} for the I_1, I_2, and I_3 treatments, respectively), but were different during the subsequent 18 days (443 ± 39, 405 ± 32, and 299 ± 13 g C m^{-2} for the I_1, I_3, and I_2/I_6 treatments, respectively), with the values decreasing with increasing W associated with the treatments (P<0.05, Table 5.2).

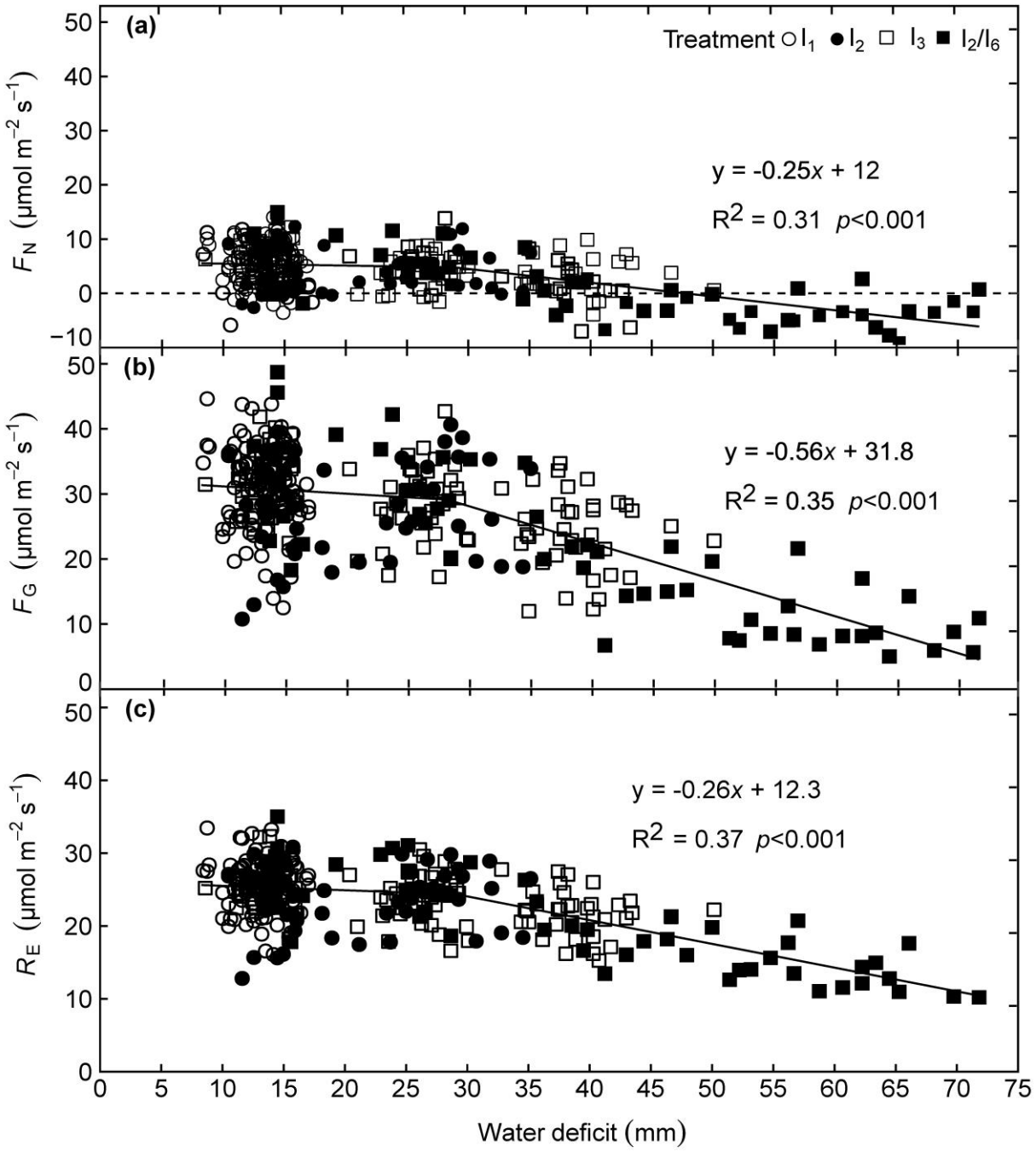

Fig. 5.2 Relationships between (a) net ecosystem CO_2 exchange rate (F_N), (b) gross carbon uptake (F_G), (c) ecosystem respiration rate (R_E) and water deficit over the 30 days. The breakpoint values for water deficit between the two lines is 29, 28, and 29 mm for F_N, F_G, and R_E, respectively. The irrigation frequencies are every 1, 2, 3 and 6 days (I_1, I_2, I_3 and I_2/I_6, respectively, n=4). Open circles, closed circles, open squares, and closed squares represent the I_1, I_2, I_3, and I_6 treatments, respectively.

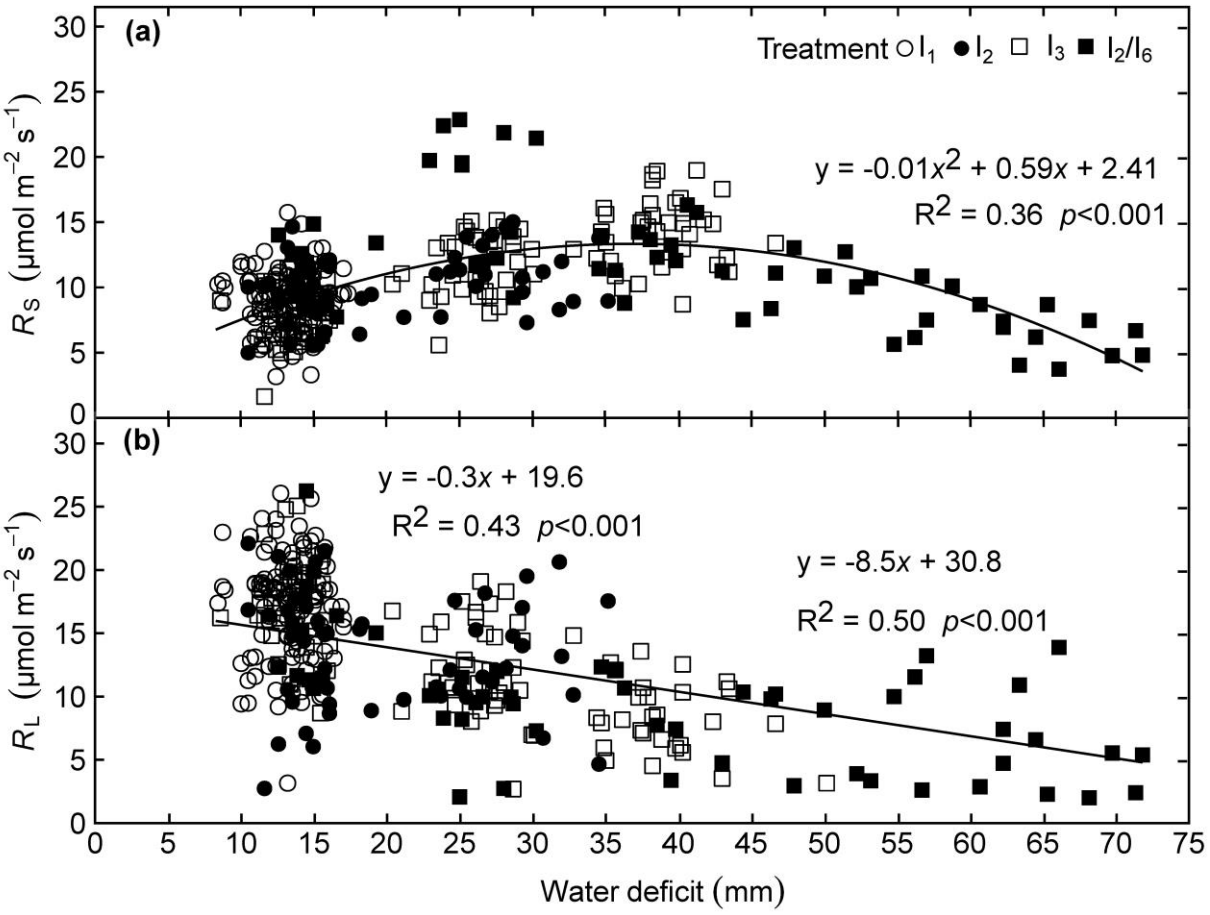

Fig. 5.3 Relationships between (a) soil respiration rate (R_S) and (b) leaf respiration rate (R_L) and water deficit over the 30 days. The breakpoint value for water deficit between the two lines is 17.1 mm for R_L. The irrigation frequencies are every 1, 2, 3 and 6 days (I_1, I_2, I_3 and I_2/I_6, respectively, n=4). Open circles, closed circles, open squares, and closed squares represent the I_1, I_2, I_3, and I_6 treatments, respectively.

Similarly, cumulative R_E declined with increasing *W* after day 12 (P<0.05, Table 5.2). In contrast, however, R_S did not increase linearly with increasing *W.* Instead, the response was best explained by a quadratic fit (R^2 = 0.36; P<0.001), with the maximum value for R_S at *W* = 37 mm (Fig. 5.3a). Cumulative values of R_S for the I_3 and the I_2/I_6 treatments were higher than those for the I_1 treatment (P<0.05), during the second 18 days period. Leaf respiration (R_L) declined linearly with increasing *W* with the threshold in the broken stick model at *W* = 17 mm, and 50% of the variation explained by the model (Fig. 5.3b). Cumulative values of R_L decreased with increasing *W* and were significantly different between treatments during days 1 to 12, with the differences increasing from days 13 to 30 (P<0.05. Table 5.2).

Table 5.2 Cumulative water deficit (*W*, mm), net ecosystem CO_2 exchange and its components (g C m^{-2}), and cumulative soil nitrous oxide (N_2O) emissions (mg N m^{-2}) over the two periods of measurements. Net ecosystem CO_2 exchange rate (F_N), gross carbon uptake (F_G), ecosystem respiration rate (R_E), leaf respiration rate (R_L), and soil respiration (R_S). The irrigation frequencies are every day, every two days, every three days and six days (I_1, I_2, I_3 and I_2/I_6, respectively). Data shown are mean ± SD, n=4. On day 13 the I_2 treatment was changed to the I_6 treatment.

Day	Treatment	Cumulative *W*	Cumulative F_N	Cumulative F_G	Cumulative R_E	Cumulative R_S	Cumulative R_L	Cumulative N_2O
1-12	I_1	160 ± 9 c	69 ± 33 a	365 ± 50 a	295 ± 18 a	87 ± 16 b	208 ± 24 a	0.21 ± 0.02 a
	I_2	248 ± 26 b	57 ± 31 a	345 ±.62 a	288 ± 34 a	122 ± 14 ab	166 ± 40 ab	0.19 ± 0.04 a
	I_3	328 ± 15 a	57 ± 27 a	329 ± 40 a	271 ± 14 a	139 ± 6 a	133 ± 14 b	0.19 ± 0.05 a
13-30	I_1	199 ± 7 c	70 ± 22 a	443 ± 39 a	373 ± 21 a	140 ± 7 a	234 ± 27 a	0.17 ± 0.02 ab
	I_3	402 ± 6 b	60 ± 16 ab	405 ± 32 b	345 ± 19 a	162 ± 11 a	183 ± 24 b	0.16 ± 0.03 b
	I_2/I_6	590 ± 27 a	18 ± 12 c	299 ± 13 c	281 ± 11 c	157 ± 12 a	124 ± 18 c	0.19 ± 0.03 a

5.4.3 Partitioning the components of soil respiration

Using the ^{13}C natural abundance isotope technique at the end of the experimental period, the values for R_H showed no significant difference between the I_1 (4.2 ± 1.1 µmol m^{-2} s^{-1}) and I_3 treatments (5.3 ± 1.2 µmol m^{-2} s^{-1}), but were much higher than that for the I_2/I_6 treatment (0.6 ± 0.5 µmol m^{-2} s^{-1}) (Fig. 5.4). R_H contributed 43 ± 8, 42 ± 8, and 8 ± 5% of R_S for the I_1, I_3, and I_2/I_6 treatments, respectively.

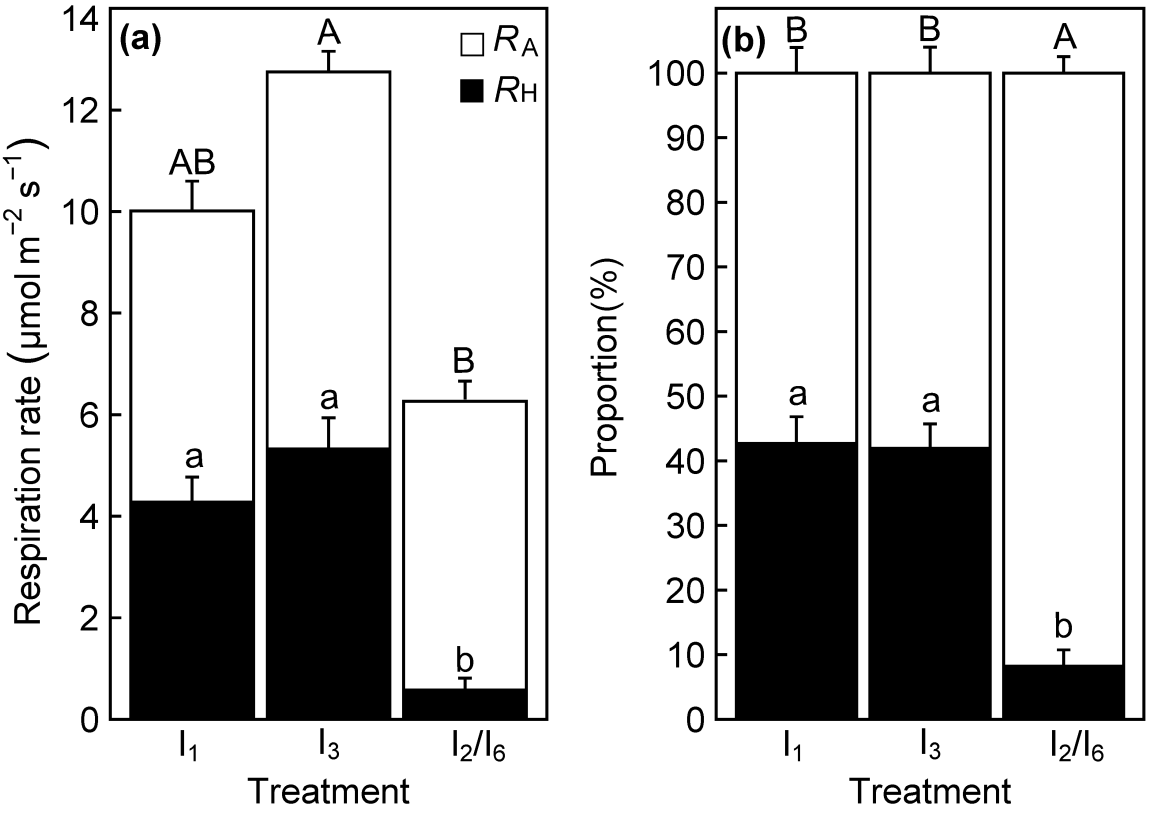

Fig. 5.4 Rates of soil heterotrophic respiration (R_H) and autotrophic respiration (R_A) (a) and their proportional contribution to R_S (b) on day 30 at the end of the measurement period when differences in water deficit between the treatments were greatest. The irrigation frequencies are every 1, 2, 3 and 6 days (I_1, I_2, I_3 and I_2/I_6, respectively). The vertical bars represent the SD of the mean (n = 4). The upper− and lower−case letters indicate significant differences in values between R_A and R_H, respectively.

5.4.4 Soil N_2O emissions

Cumulative N_2O emissions were slightly lower for the I_3 treatment than in the I_2/I_6 treatment in the period from days 13 to 30 but otherwise there were no significant differences between the treatments (Table 5.2). There was a weak relationship between decreasing soil N_2O emissions with

increasing cumulative *W*, which was described by an exponential fit where N_2O emissions = $1.24 \times e^{-0.02 \times W}$ (R^2 = 0.05, P<0.001), but this only explained 6% of the variability in N_2O emissions that ranged from 0.1 to 6.9 mg N m^{-2} h^{-1}.

5.5 Discussion

5.5.1 Response of carbon balance components to irrigation frequency

The threshold values of *W* for the broken stick model below which F_N, F_G and R_E remained constant at maximum values were similar regardless of treatment, and occurred at a fraction of total water holding capacity for the soils of between 0.4 and 0.5, suggesting similar threshold values for photosynthesis and ecosystem respiration. The lack of any treatment differences in cumulative F_N, F_G and R_E during the first 12 days demonstrated that these processes are insensitive to mild increases in *W* up to 30 mm. This coincides closely with the breakpoints between no effects and decreases in both photosynthesis and leaf expansion with increasing *W* for a range of crops and soil types (Sadras and Milroy, 1996). Such an approach with a decrease in plant growth at *W* below a threshold value has long been used to manage irrigation scheduling for field crops (Monteith et al., 1986).

Based on a meta−analysis of data across biomes, Zhou et al. (2016) concluded that drought resulted in decreased net photosynthesis by 25% but also decreased R_E by 18%. The same study showed that irrigation increased plant net photosynthesis by 34% and increased R_E by 26%. With our values of F_N decreasing after the threshold value of *W*, we also provided evidence that R_E was less sensitive to drying soils than the sensitivity of F_G, so much so that our mesocosms started losing C (negative F_N) at higher values of *W*. However, in contrast to another study (Zhang et al., 2019), we did not find a positive relationship between changes in aboveground net primary production and soil water availability, with constant aboveground biomass produced across treatments. The C allocated to the aboveground plant component must therefore have remained unchanged with increasing *W*, despite decreasing net ecosystem C uptake. This was confirmed by the observation that reductions of F_G and R_L were similar in response to increasing *W* (with decreases in cumulative values between the I_1 and I_2/I_6 treatments of 67 and 60%, for F_G and R_L, respectively).

The decline in R_E with increasing *W* resulted from different responses of R_L and R_S (Fig. 5.3, Table 5.2). We attribute this difference in the response of R_L and R_S to be the result of a complex interaction between autotrophic and heterotrophic components of the soil, leading to the observed quadratic response of R_S. From comparative measurements at 19 temperate grassland sites, Burri et al. (2018) concluded that the response of decreasing R_S to drought was independent of aboveground productivity but was strongly linked with below−ground C allocation. We argue that increasing

relative C allocation to promote root growth (van Wijk, 2011) and associated respiration rates (Meier and Leuschner, 2008) can explain the changes in R_A with increasing W.

We showed that the decreases in R_H (23%) with increasing W across the treatment extremes were greater than the change in R_A (10%) (Fig. 5.4). This finding is in contrast with the general trend in grasslands found from the meta−analysis by Zhou et al. (2016) where irrigation increased R_A and R_H by 21 and 28%, respectively. Heinemeyer et al. (2012) also reported that R_A was relatively stable throughout the 3−month period in a temperate grassland during summer. A shift in C allocation belowground may also affect R_H due to changes in priming of SOM (Kuzyakov et al., 2000). However, both Fuchslueger et al. (2014) and Karlowsky et al. (2018) used a ^{13}C tracer to show that drought strongly reduced the availability of root exudates for microbial activity. Several studies have shown that R_H is sensitive to increasing W, particularly in semi−arid systems (Chen et al., 2009) when the balance between water content and oxygen availability become suboptimal for microbial activity. Decreasing R_H in our study shows that respiratory losses of soil C declined with decreasing irrigation frequency. However, this possibly masked the effects of pulses of respired CO_2 occurring rapidly after re−wetting soil. A meta−analysis including 1495 observations from 60 studies found that the respiration pulse after rewetting completely compensated for the decrease in respiration during the drying phase, so that cumulative respiration was not significantly different between the control (constant water supply) and drought treatments (Canarini et al., 2017).

5.5.2 Soil N_2O emissions

Soil N_2O emissions measured in this study ranged from 0.1 to 6.9 mg N m^{-2} h^{-1} and were lower than those typically measured in irrigated, ungrazed grassland in field conditions (Saggar et al., 2010). Oxygen availability is the main driver of denitrification and is strongly regulated by soil water content (Linn and Doran, 1984; Owens et al., 2016). In this study, even the range in WFPS of 36 to 54% did not result in conditions that were sufficiently anaerobic to support denitrification (Linn and Doran, 1984). Thus, it is likely that N_2O emissions were dominated by nitrification and the lack of differences between the treatments is consistent with earlier findings for freely draining soils (Owens et al., 2016).

5.6 Conclusions

The findings from this grassland study show that decreases in ecosystem net C balance (F_N), with increasing cumulative soil water deficit (W), were moderated by the offset between a strong decrease in gross C uptake by plants (F_G) and a less sensitive response in ecosystem respiration (R_E).

However, aboveground biomass production remained constant, due to similar responses of F_G and respiratory losses from leaves (R_L) to increasing *W*. Cumulative R_S did not increase with treatment extremes, but the use of a $\delta^{13}C$ natural abundance stable isotope technique showed maintained root respiration (R_A) offset by a strong decrease in the fraction of respiration derived from SOM decomposition (R_H). This suggests that decreasing irrigation frequency could lead to a reduction in soil C losses from SOM decomposition while keeping C inputs to the soil constant (with supporting evidence from small changes in R_A), with no change in aboveground biomass. The findings highlight the importance of incorporating the relative response of the components of C balance to increasing *W* in models that predict C losses from soils. Furthermore, there were no differences in soil N_2O emissions in the absence of high inputs of N in this well−drained, aerobic soil. The findings therefore demonstrate that changes to irrigation scheduling could be used to minimise soil C losses but that this is unlikely to affect N_2O emissions with low N inputs on well−drained soils.

Supplemental Table S5.1 The $\delta^{13}C$ values (‰) for roots and soil samples and CO_2 evolved from the soil surface and CO_2 partial pressure ([CO_2] µmol mol^{-1}) from the undisturbed soil surface and for the three treatments at the end of the experimental period. The irrigation frequencies are every day, every two, three days and every six days (I_1, I_2, I_3 and I_2/I_6, respectively, n=4). On day 13, the I_2 treatment was changed to the I_6 treatment.

Treatment	Replicate	[CO_2]	Root	Soil	Soil surface
I_1	1	416.79	–13.59	–27.94	–20.5
	2	204.14	–13.54	–28.26	–18.35
	3	229.37	–13.75	–28.17	–19.43
	4	244.06	–13.70	–28.15	–21.01
I_3	1	345.24	–13.89	–27.94	–20.12
	2	408.03	–13.85	–28.06	–18.31
	3	242.71	–13.93	–28.33	–20.07
	4	212.66	–14.03	–28.83	–21.34
I_2/I_6	1	204.31	–14.04	–28.21	–14.82
	2	221.68	–13.96	−28.19	–14.41
	3	264.09	–13.76	–28.16	–15.17
	4	262.07	–14.13	–28.30	–16.19

Chapter 6 Key findings and future research recommendations

6.1 Key findings

Based on the results from the previous chapters that included two laboratory incubation experiments and a pot trial, this chapter briefly summaries key findings from the thesis, and the implications of these before suggesting some future research directions.

Experiment 1 (Chapter 3) involved applying C substrate, to three NO_3^- amended soils, that were held at three levels of soil matric potential. Emissions of N_2O and CO_2 were monitored for 14 days with N_2 emissions determined on days three and fourteen. Key findings included:

- Addition of glucose or acetate, in the presence of added NO_3^- increased CO_2, N_2O and N_2 emissions.
- When N_2O emissions were dominant to N_2 emissions, ca. 3 days after substrate addition, acetate enhanced N_2O reduction to N_2, lowering the N_2O:(N_2O+N_2), in three grassland soils when compared to glucose.
- The effect of C substrate type on the N_2O:(N_2O+N_2) ratio at day 3 was consistent in all soils, but only if the interaction between soil type and soil matric potential resulted in conditions suitable for denitrification (D_P/D_O < ~0.006).
- By day 14 denitrification emissions were dominated by N_2 emissions, under both acetate and glucose, to the point where N_2O emissions under C substrate addition were lower than in the water-only control. However, the N_2 emissions at this time, under acetate, were 2-fold higher when compared with glucose: denitrification rate was enhanced under acetate on day 14.
- The 2-fold higher denitrification rate under acetate at day 14 was soil specific. It occurred only in the LU and LD soils, not the AD soil.
- Emissions of CO_2 reached steady state at all soil moisture contents in the LU and LD soils but not for all moisture contents in AD the soil.

Experiment 1 indicated that differences in soil gaseous emissions might occur as a consequence of soil priming. Thus, Experiment 2 (Chapter 4) involved applying three ^{13}C labelled substrates (acetate, glucose and butyrate), in conjunction with ^{15}N labelled NO_3^-, to three soils that were held at three levels of soil matric potential. Emissions of N_2O and CO_2 and their respective ^{15}N and ^{13}C enrichments were measured on day 3. Key findings included:

- Application of isotopically labelled substrates and the measurement of $^{13}CO_2$ and $^{15}N_2O$ emissions enabled the quantification of C substrate priming effects on CO_2 and N_2O emissions.
- The magnitude of the C substrate priming effect and its direction (positive or negative) depended on C substrate type: positive, neutral and negative priming occurred following acetate, glucose, and butyrate addition, respectively.
- Emissions of CO_2 and N_2O from soils with added glucose were higher than those from soils treated with acetate or butyrate respectively.
- Carbon substrate addition increased SOM derived N_2O emissions in the presence of exogenous N.
- The contribution of SOM to the N_2O emissions was relatively low from soils with butyrate addition when compared with acetate or glucose. However, there was no clear relationship between the priming of SOM and SOM derived N_2O emissions.

In the third experiment (Chapter 5), C_4 grassland plants were grown in mesocosms with C_3 soil to determine the effects of irrigation frequency (either 1, 2, or 3 days for 12 days, after which the treatment where watering was occurring at every 2 days was changed to watering every 6 days), on CO_2 and N_2O emissions.

- Gross C uptake (F_G) by the plants, and ecosystem respiration (R_E) showed similar decreases with increasing cumulative water deficit.
- Above−ground plant respiration (R_L) was more sensitive than below−ground respiration (R_S) to increasing water deficit.
- At the end of the experiment when differences in cumulative water deficits were greatest, differences in soil respiration (R_S) were attributable to a decreasing contribution from SOM decomposition (R_H) and an increasing contribution from roots (R_A), with increasing cumulative soil water deficit.
- There were no detectable changes in N_2O emissions between the water deficit treatments under the experimental conditions.
- The findings highlight the importance of soil microbial processes in regulating soil respiration in irrigated grassland and the need to incorporate these processes in models that predict C losses from soils. Changes to the scheduling of irrigation could reduce CO_2 emissions and SOM decomposition but not N_2O emissions in conditions of moderate to high water deficits.

The findings from these experiments further demonstrate the intricate interactions between C and N substrates, and soil moisture, on the emissions of CO_2, N_2O and N_2.

The first requirement for denitrification of nitrate, regardless of adequate C and N substrate supply, is a near zero soil oxygen concentration ($D_P/D_O < \sim 0.006$). As the data showed this prerequisite may not occur if the soil is too freely draining (e.g. AD soil at -7 kPa).

The value of D_P/D_O also determined the magnitude of CO_2 emissions, with higher emissions occurring as D_P/D_O increased in the current studies. Hence, the soil physical characteristics initially determine emission potentials. In conjunction with the soil physics occurs the soil biology, which was not explored in the current studies. However, given the results where the findings show changes in the N_2O:(N_2O+N_2) ratio over time, and differences in the magnitude of CO_2 and denitrification emissions over time, and the time taken to reach steady state differing between soils, the results demonstrate that a detailed examination of microbial dynamics using molecular methods is warranted to better explore C substrate type effects on CO_2 and denitrification emissions. It was postulated that the physical history or management of the three soils may have generated microbial communities not equally responsive to substrate additions and in conjunction with priming studies there emerges a clear research direction to advance this knowledge as indicated below.

If soil physical conditions induce a water deficit then the interaction between N and C becomes less fraught with emissions dominated by water deficit effects on CO_2. Decreases in net ecosystem CO_2 exchange (F_N), with increasing cumulative soil water deficit, were moderated by the offset between a strong decrease in plant C uptake (F_G) and a less sensitive response in ecosystem respiration (R_E). Thus, the modelled grassland started to lose C as indicated by the negative net ecosystem CO_2 exchange (F_N). However, aboveground biomass was constant across treatments due to similar responses of net ecosystem CO_2 exchange (F_N) and leaf respiration (R_L) to increasing water deficit. The findings open an opportunity to forecast trade−offs in soil C losses, gross primary production, and soil N_2O emission in irrigated grasslands. But further data in other grassland systems, measuring these independent emissions, are needed in order to build a picture of the ecosystem status. For example, it is unclear how the data from the current study using a C_4 plant will apply to temperate C_3 grassland species.

However, the data suggest that decreasing irrigation frequency could lead to a reduction in soil C losses from SOM (R_H) while C inputs to the soil remain relatively constant. The findings highlight the importance of the microbial decomposition of soil organic matter in response to changes in soil water content.

6.2 Future research recommendations

1. Experiment 1 demonstrated that the C substrate present and the duration of the denitrification event both affected the N_2O:(N_2O+N_2) ratio and the amount of denitrification. If plant breeding strategies are aligned with screening for or designing for specific root exudate compounds then it is clear that further research is required to better understand the net result on denitrification losses over time. Experiments are required to extend current knowledge of C substrate type effects on the N_2O:(N_2O+N_2) ratio and N_2 emissions in particular.
2. A component of such substrate studies should try and identify the mechanism responsible for the acetate induced reduction in the N_2O:(N_2O+N_2) ratio when acetate is applied.
3. Similarly, a further component of such studies should aim to better understand the circumstances as to how fermentative micro-organisms might compete with denitrifiers for C substrate, and the ensuing effect(s) this may have on denitrification emissions and the N_2O:(N_2O+N_2) ratio. For example, stable-isotope-priming may be a tool that could be applied.
4. Consideration must also be given to understanding the potential side effects of selecting system inputs that favour reduced N_2O emissions (e.g. plants bred to secrete high volumes of acetate) but which might have the potential for higher soil priming effects.
5. Addition of C substrates initiates priming of SOM, but the chemical nature of different substrates affects the direction and magnitude of priming. Under conditions of moderate to high soil water content, there was no clear relationship established between priming of SOM and N_2O emissions. This should be examined further with respect to the N_2O:(N_2O+N_2) ratio given that the 'primed' N_2O may be rapidly transformed to N_2.

Further studies are also recommended to better understand the effects of improved irrigation management on soil C losses. It was observed that as water deficit increased R_A, as a percentage of R_S, increased, while R_H also decreased. The relative change in the magnitude of these emissions should be studied further in terms of New Zealand's dominant grassland species (perennial ryegrass (*Lolium perenne* L.)), and with respect to the increasing popularity of 'regenerative agriculture' where multiple grassland species are promoted. The focus of such studies could examine the potential for differences in the R_A/R_H ratio with respect to water deficit across multiple species.

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Appendix A

A.1 Soil core preparation

Air−dried soil was sieved to < 2 mm and repacked into stainless steel cylinders (Fig. A1) for Experiment 1 (section 3.3.1) and Experiment 2 (section 4.3.1) to set values of bulk density, ρ_b, from

$\rho_b \times (V_S + \theta_g \times \rho_b \times V_S) / M_S$

Ms is the mass of dry soil, V_S is the volume of the soil and θ_g is gravimetric water content.

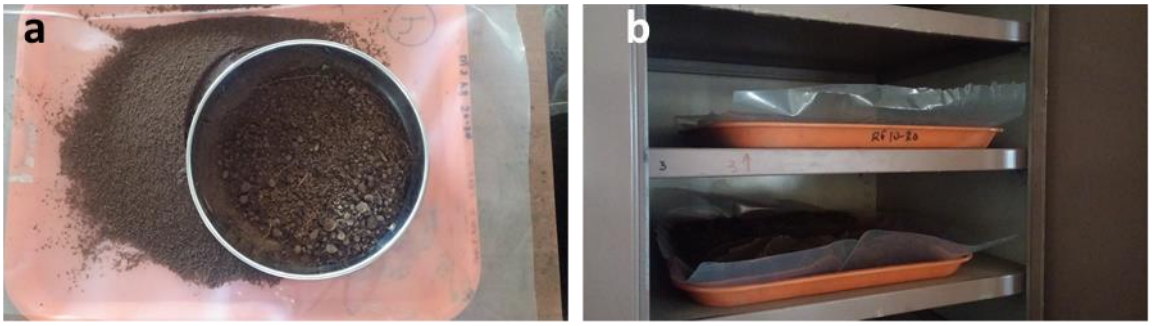

Fig. A1 Soil preparation: the process of sieve (a) and air−dry (b).

A snug−fitting tool (Fig. A2) was used to compress different masses of soil into the set volume for each value of ρ_b in four stages each 10 mm depth. The bottom of each cylinder was covered with a fine nylon mesh to prevent any soil loss.

Fig. A2 Packing the soil in the steel cylinders (a) using a four stage process (b) and (c) the soil removed at the end of the experiments.

A.2 Preparation of mesocosms

For the mesocosms in Experiment 3 (section 5.3.1), topsoil was collected from a long−term grazed grassland site dominated by perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) from the Lincoln University Demonstration Farm and a constant mass was placed to a set volume to give a constant bulk density in each PVC cylinders 200 mm diameter and 300 mm depth. Fifteen seeds of the C_4 Bermuda grass (*Cynodon dactylon* L.) (10 g m^{-2}, PGG Wrightson Seeds, Christchurch, NZ) were sown in the annulus outside each PVC collar. The mesocosms were then placed in a controlled environment cabinet (Model HGC 1514, Weiss Gallenkamp, UK) set at constant conditions of air temperature 25°C, photoperiod 16 h at photosynthetically active irradiance (400−700 nm) of 600−650 µmol m^{-2} s^{-1} and relative humidity 70%.

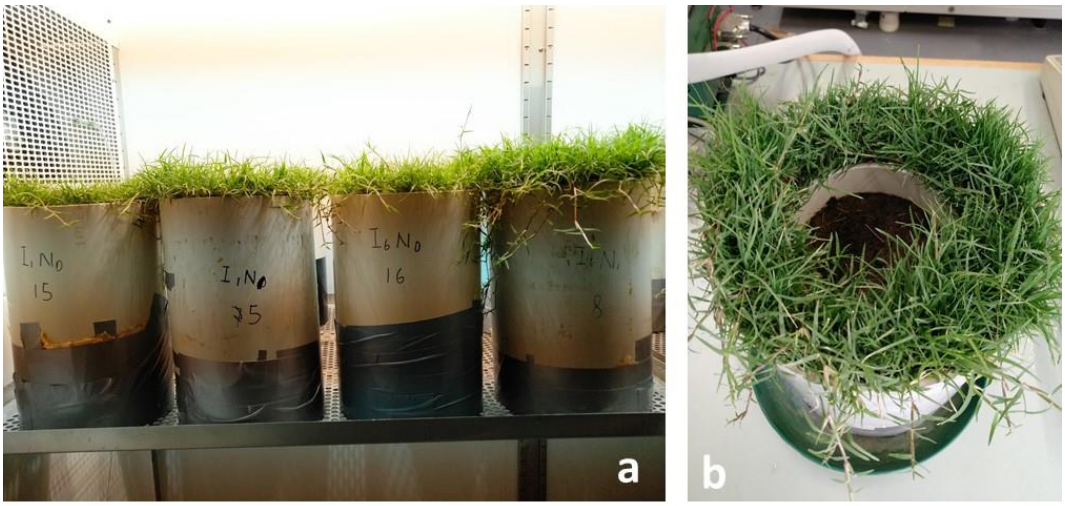

Fig. A3 Mesocosms in controlled conditions (a) showing the annulus of Bermuda grass growing around the central collar to facilitate partitioning of the components of soil respiration (b).

A.3 Tension tables

Tension tables (Fig. A4) as described by Romano et al. (2002) were used in Experiment 1 (section 3.3.1) and Experiment 2 (section 4.3.1).

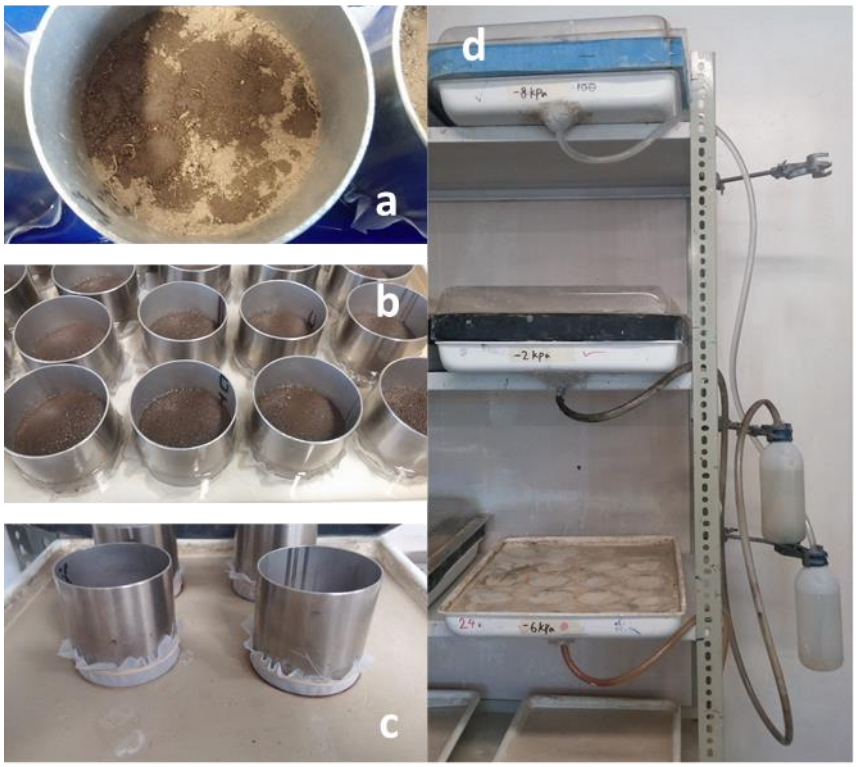

Fig. A4 Pre−soaking (a, b) and equilibration of soil core on the tension tables (c, d).

A.4 Measurements of CO_2 emissions, N_2O emissions and the components of carbon balance

Rates of CO_2 emissions were measured in Experiment 1 (section 3.3.3) and Experiment 3 (section 5.3.2) using a static chamber placed on top of the soil cores removed from the tension tables and attached to an automated soil respiration system (Model LI−8100, LI−COR Inc., Lincoln, Nebraska, USA).

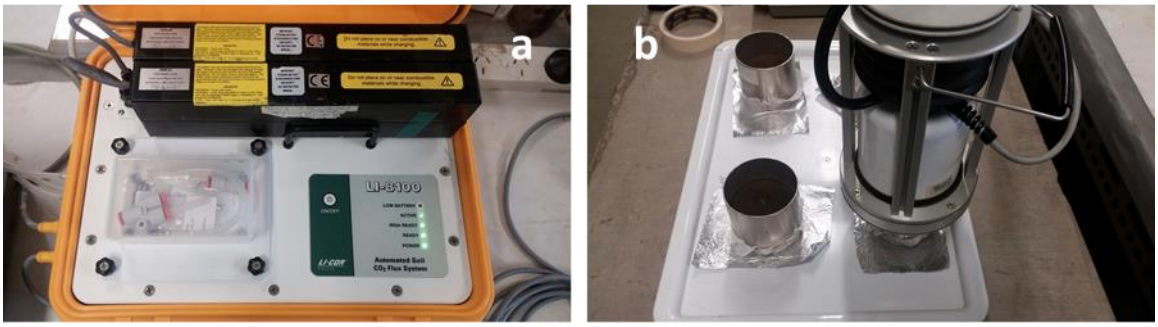

Fig. A5 Instrumentation (a) and the chamber (b) used for measurements of CO_2 emissions.

Net ecosystem CO_2 exchange for the mesocosms in Experiment 3 (section 5.3.2) was measured under full irradiance using a purpose-built chamber placed on the top of each mesocosm for a period of 2 min as described by (Moinet et al., 2016b) (Fig. A5). Subsequently, the measurement was repeated to

estimate ecosystem respiration (R_E) by excluding light using a dark cloth placed over the mesocosm and chamber. The rate of soil respiration (R_S) was measured by placing a chamber from an automatic soil respiration system (Model LI−8100, LI−COR Inc., Lincoln, Nebraska, USA) on the central collar in each mesocosm.

Fig. A6 Measurements of net ecosystem CO_2 exchange (a), ecosystem respiration (b), soil respiration (c), and gas sampling to calculate nitrous oxide emissions (d).

For calculations of N_2O emissions in Experiment 3 (section 5.3.2), the central collar was covered and sealed with a gas−tight fitted with a two−way stopcock. A 25G hypodermic needle was used to remove gas samples (10 mL) for measurements of N_2O concentrations (Fig. A6).

For Experiment 1 (section 3.3.3) and Experiment 2 (section 4.3.2) a different set up was used to collect N_2O samples (Fig. A7). The soil cores were removed from the tension tables and placed into 1L stainless steel tins equipped with gas−tight lids fitted with rubber septa (Fig. A7). Gas samples (10 mL) were removed after sealing the tin using a 20 mL glass syringe fitted with a three−way stopcock and a 25G hypodermic needle.

Fig. A7 Gas−tight jars and syringes used to sample headspace N_2O concentrations.

Measurements of $\delta^{13}C R_S$, from each treatment in Experiment 3 (section 5.3.3), were made by collecting air respired from the soil surface using a partially automated open−chamber system described by (Midwood et al., 2008; Moinet et al., 2016b) (Fig. A8). The chambers were placed on the central collar in each mesocosm and CO_2 free air was supplied at a variable rate to maintain the CO_2 partial pressure inside the chamber at 500 µmol mol^{-1} (Fig. A8a). After an equilibrium rate was constant for 90 minutes, 500 mL of respired air was collected into gas−tight sample bags (Tedlar® Keika Ventures, Chapel Hill, NC, USA) that were flushed twice with CO_2 free air and evacuated prior to use (Fig. A8b). The gas was analysed for $\delta^{13}C$ values using a cavity ringdown spectrometer (model G2121−I, Picarro Inc., Santa Clara, CA, USA) (Fig. A8c). The reference gas was calibrated with Pee−Dee−Belemnite (PDB) certified standard for isotope signature calculations.

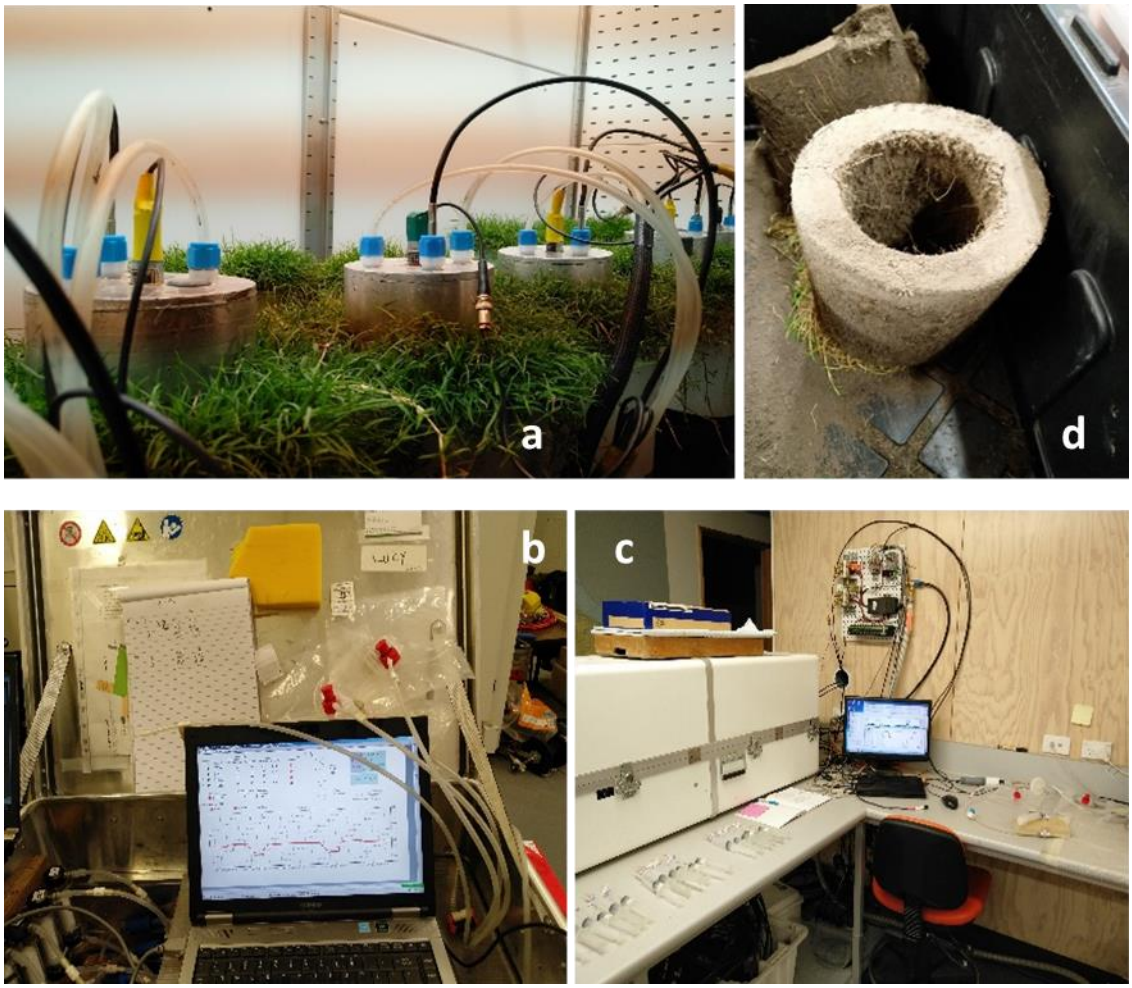

Fig. A8 Analysis of air samples for isotopic abundance of CO_2 (a–c) and destructive sampling of a soil and root sample removed from the mesocosms (d).

A.5 Soil relative gas diffusivity

Measurements of soil relative gas diffusivity (D_P/D_O) for Experiment 1 (section 3.3.3) were made using the system shown in Fig. A9. The gas diffusion chamber was engineered following Rolston and Moldrup (2002).

Fig. A9 System for the measurements of soil relative gas diffusivity (a), reference gases for calibration (b) and diffusion chamber with an oxygen sensor inside (c).